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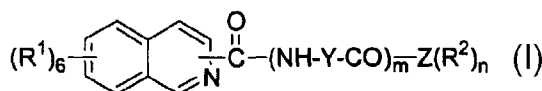
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(54) Title: ISOQUINOLINE COMPOUNDS HAVING ANTIINFECTIVE ACTIVITY



(57) Abstract: Isoquinoline compounds having the formula where R₁, R₂, Y, m, n, and Z are as defined herein, bind to the minor groove of DNA and have antifungal and antibacterial activity.

ISOQUINOLINE COMPOUNDS HAVING ANTIINFECTIVE ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[01] This application claims the benefit of U.S. Ser. Nos. 60/333,830, filed Nov. 27, 2001, and 60/298,206, filed Jun. 13, 2001; the disclosures of which are incorporated
5 herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[02] This invention was made with Government support under Grant No. N65236-99-1-5427 awarded by the Space and Naval Warfare Systems Command. The Government
10 has certain rights in this invention

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[03] This invention relates to isoquinoline compounds, in particular ones binding to nucleic acids and having anti-bacterial properties, and methods for their use.

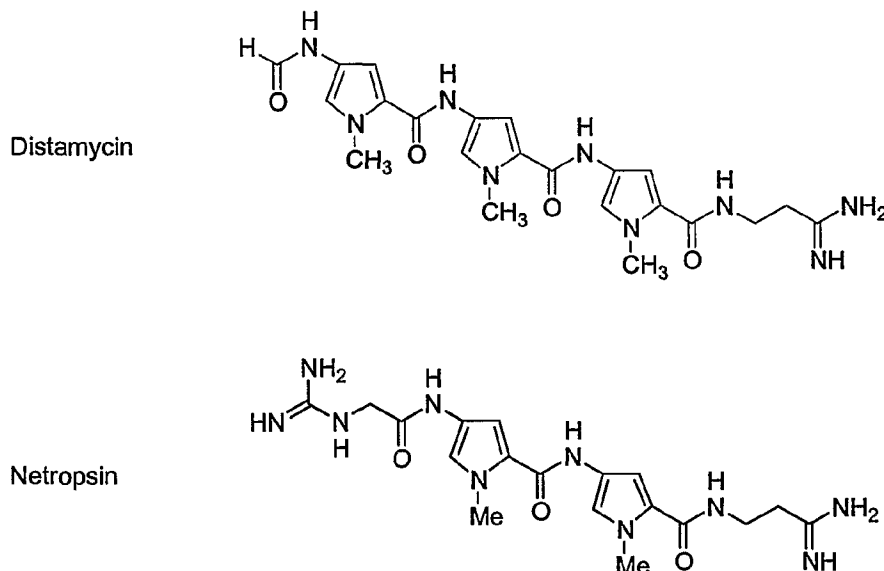
15 DESCRIPTION OF RELATED ART

[04] A number of naturally occurring or synthetic compounds bind to double stranded nucleic acid, especially double stranded DNA ("dsDNA"). Some bind to the major groove, while others bind to the minor groove. Still others intercalate between adjacent base pairs. Combination binding modes are known, in which a compound has binding
20 interactions with more than one nucleic acid site.

[05] It has been proposed to use dsDNA binding compounds to regulate the expression of genes for medical purposes. If a disease is characterized by the overexpression or undesired expression of a gene (e.g., an oncogene), in principle the disease can be treated by suppressing wholly or partially the gene's expression via the binding of a compound to
25 the gene or a promoter site thereof and interfering with transcription. Infections by pathogens such as fungi, bacteria, and viruses can be treated with compounds that interfere with the expression of genes essential for the pathogen's proliferation. Or, in a

disease characterized by non- or under-expression of a beneficial gene, the expression of the beneficial gene can be up-regulated with a compound that binds to the binding site of a repressor, displacing the repressor.

[06] The natural products distamycin and netropsin represent a class of DNA-binding compounds that has been studied over the years:

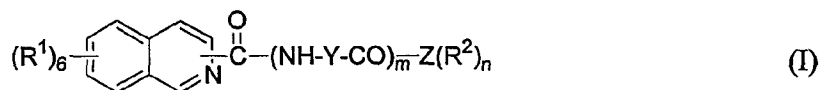


Structurally, distamycin and netropsin are heteroaromatic polyamides, having as their core structural motif N-methylpyrrole carboxamide residues. They bind to the minor groove, their crescent molecular shapes providing a conformational fit within the groove. The binding occurs with a preference for A,T rich dsDNA tracts.

[07] Many heteroaromatic polyamides have been synthesized elaborating on the distamycin/netropsin motif, with the objective of enhancing or varying biological activity, increasing binding affinity to dsDNA, and/or improving specificity in base pair sequence recognition. See Bailly et al., *Bioconjugate Chemistry* 1998, 9 (5), 513-538, and Neidle, *Nat. Prod. Rep.* 2001, 18, 291-309. The use of synthetic heteroaromatic polyamides in therapeutics has been proposed, for example, in Dervan et al., US 5,998,140 (1999); Dervan et al., WO 00/15209 (2000); Dervan, WO 00/15773 (2000); and Gottesfeld et al., WO 98/35702 (1998).

BRIEF SUMMARY OF THE INVENTION

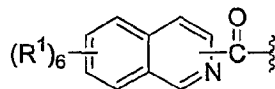
[08] This invention provides isoquinoline compounds having the formula



including the pharmaceutically acceptable salts thereof.

- [09] Each R^1 is independently H, F, Cl, Br, I, CN, OH, NO₂, NH₂, a substituted or unsubstituted (C₁-C₁₂)alkyl group, a substituted or unsubstituted (C₁-C₁₂)alkoxy group, or
 5 a substituted or unsubstituted (C₁-C₁₂)heteroalkyl group.

- [10] Each Y is independently a branched or unbranched, substituted or unsubstituted (C₁-C₅)alkylene group or a substituted or unsubstituted, aromatic or heteroaromatic ring system, wherein the ring system has a 5- or 6-member aromatic or heteroaromatic ring or fused 6,6 or 6,5 aromatic or heteroaromatic rings, with the proviso that at least one Y is a
 10 substituted or unsubstituted aromatic or heteroaromatic ring system. Preferably, at least one Y is a 5- or 6-member heteroaromatic ring. More preferably, Y in the moiety – (NH-Y-CO)- immediately adjacent to



is a 5- or 6-member heteroaromatic ring.

- 15 [11] Subscript m is an integer from 1 to 25, inclusive, preferably from 1 to 6, more preferably from 2 to 4.

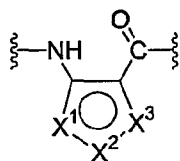
[12] Z is either O or N, with n being 1 if Z is O and 2 if Z is N.

[13] Each R^2 is independently H, a substituted or unsubstituted (C₁-C₁₂)alkyl group, or a substituted or unsubstituted (C₁-C₁₂)heteroalkyl group.

- 20 [14] Compound (I) has a basic group having a pK_b of 12 or less or a quaternized nitrogen group.

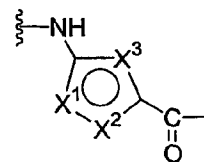
[15] Preferably, each moiety –(NH-Y-CO)- is independently selected from the group consisting of:

- (a) moieties M^1 having the formula



(IIa)

or



(IIb)

wherein one of X^1 , X^2 , and X^3 is a ring vertex selected from the group consisting of -O-, -S-, and -NR²-, and the other two of X^1 , X^2 , and X^3 are ring vertices selected from the group consisting of =N- and =CR¹-;

- 5 (b) moieties M² having the formula



(III)

wherein x is 0 or 1 and each R¹⁵ is independently H, OH, NH₂, or F;

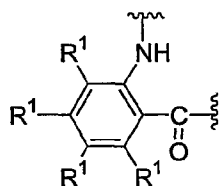
- (c) moieties M³ having the formula



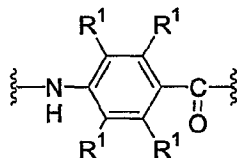
(IV)

- 10 wherein each L is independently a divalent moiety separating -NH- and -(C=O)- by 3 or 4 atoms; and

- (d) moieties M⁴ having the formula

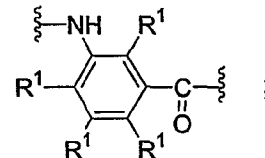


(Va)



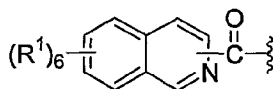
(Vb)

or



(Vc)

- [16] Preferably, at least one moiety -(NH-Y-CO)- is a moiety M¹. More preferably,
15 the moiety -(NH-Y-CO)- immediately adjacent to the residue



is a moiety M¹.

- [17] In the preceding formulae, R¹ and R² are as previously defined.

[18] Preferably, R¹ is hydrogen, halogen (F, Cl, Br, or I), a (C₁-C₅)alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, pentyl, and the like, a (C₁-C₅)alkoxy group such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, and the like, hydroxy, or cyano. Preferably, each R² is H or a (C₁-C₅)alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, pentyl, and the like.

BRIEF DESCRIPTION OF THE DRAWING(S)

[19] Figs. 1a through 1f and 2a through 2c show the structures of exemplary compounds according to this invention.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations and Definitions

[20] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* C₁-C₁₀ means one to ten carbons). Examples of saturated hydrocarbon radicals include groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butyne, and the higher homologs and isomers.

[21] The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified by -CH₂CH₂CH₂CH₂-. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having six or fewer carbon atoms.

[22] The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

[23] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and from one to three heteroatoms selected from the group consisting of O, N, Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S may be placed at any interior position of the heteroalkyl group. The heteroatom Si may be placed at any position of the heteroalkyl group, including the position at which the alkyl group is attached to the remainder of the molecule. Examples include -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -CH=CH-O-CH₃, -Si(CH₃)₃, -CH₂-CH=N-OCH₃, and -CH=CH-N(CH₃)-CH₃. Up to two heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified by -CH₂-CH₂-S-CH₂CH₂- and -CH₂-S-CH₂-CH₂-NH-CH₂-. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied.

[24] The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

[25] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom.

Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C₁-C₄)alkyl" is meant to include trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[26] The term "aryl" means, unless otherwise stated, a polyunsaturated, typically aromatic, hydrocarbon substituent which can be a single ring or multiple rings (up to three rings) which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from zero to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

[27] For brevity, the term "aryl" when used in combination with other terms (*e.g.*, aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (*e.g.*, benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (*e.g.*, a methylene group) has been replaced by, for example, an oxygen atom (*e.g.*, phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

[28] Each of the above terms (*e.g.*, "alkyl," "heteroalkyl," "aryl" and "heteroaryl") are meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[29] Substituents for the alkyl, heteroalkyl, aryl, and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be a variety of groups selected from: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''',

-OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'' and R''' each independently refer to hydrogen, unsubstituted (C₁-C₈)alkyl and heteroalkyl, unsubstituted aryl, aryl substituted with 1-3 halogens, unsubstituted alkyl, alkoxy or thioalkoxy groups, or aryl-(C₁-C₄)alkyl groups. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups such as haloalkyl (e.g., -CF₃ and -CH₂CF₃) and acyl (e.g., -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like). Preferably, the substituted alkyl and heteroalkyl groups have from 1 to 4 substituents, more preferably 1, 2 or 3 substituents. Exceptions are those perhalo alkyl groups (e.g., pentafluoroethyl and the like) which are also preferred and contemplated by the present invention.

[30] Similarly, substituents for the aryl and heteroaryl groups are varied and are selected from: -halogen, -OR', -OC(O)R', -NR'R'', -SR', -R', -CN, -NO₂, -CO₂R', -CONR'R'', -C(O)R', -OC(O)NR'R'', -NR''C(O)R', -NR''C(O)₂R', -NR'-C(O)NR''R''', -S(O)₂R', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂NR'R'', -N₃, -CH(Ph)₂, perfluoro(C₁-C₄)alkoxy, and perfluoro(C₁-C₄)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'' and R''' are independently selected from hydrogen, (C₁-C₈)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C₁-C₄)alkyl, and (unsubstituted aryl)oxy-(C₁-C₄)alkyl.

[31] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-(CH₂)_q-U-, wherein T and U are independently -NH-, -O-, -CH₂- or a single bond, and q is an integer of from 0 to 2. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and B are independently -CH₂-, -O-, -NH-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond, and r is an integer of from 1 to 3. One of the single bonds of the new ring so

formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CH_2)_s-X-(CH_2)_t-$, where s and t are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or -S(O)₂NR'-. The substituent R' in -NR'- and -S(O)₂NR'- is selected from hydrogen or unsubstituted (C₁-C₆)alkyl.

[32] As used herein, the term "heteroatom" is meant to include oxygen (O), nitrogen (N), sulfur (S) and silicon (Si).

[33] The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogen-carbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogen-sulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, ascorbic, propionic, isobutyric, maleic, malonic, lactic, malic, glutamic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, lactobionic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge, S.M., et al, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, **66**, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[34] The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

[35] In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

[36] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[37] Certain compounds of the present invention possess asymmetric carbon atoms (chiral centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

[38] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

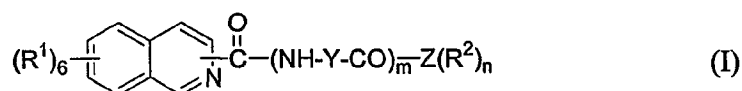
[39] In the discussions below, reference is made to dsDNA as the nucleic acid, but it is to be understood that the invention is not limited to dsDNA and is applicable to other nucleic acids, i.e., ribonucleic acid.

Compounds

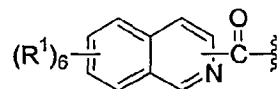
5 [40] Compounds (I) of this invention are poly- or oligoamides having an isoquinoline carboxamide unit and aliphatic, aromatic, and/or heteroaromatic carboxamide units.

[41] Polyamides (I) are DNA-binding compounds, which bind to the minor groove of dsDNA. Different binding modes are possible. In the simplest mode, often referred to as the 1:1 binding mode, a single polyamide molecule fits in the channel formed by the
 10 minor groove. In what is referred to as the 2:1 binding mode, two polyamide molecules fit side-by-side in the minor groove, preferably aligned in an antiparallel manner (i.e., with one polyamide being aligned N-to-C and the other polyamide being aligned C-to-N, where "C" and "N" refer to the carboxy and amino termini, respectively of the polyamides). Lastly, in what is referred to as a "hairpin" binding mode, a single polyamide
 15 molecule that has a more or less centrally positioned flexible moiety (i.e., a moiety M^3 , as discussed in greater detail hereinbelow) folds around itself to adopt a hairpin conformation when it is bound to the minor groove, so that a first portion of the polyamide at one side of the hairpin turn is adjacent to a second portion of the polyamide at the other side of the hairpin turn.

20 [42] In formula (I)

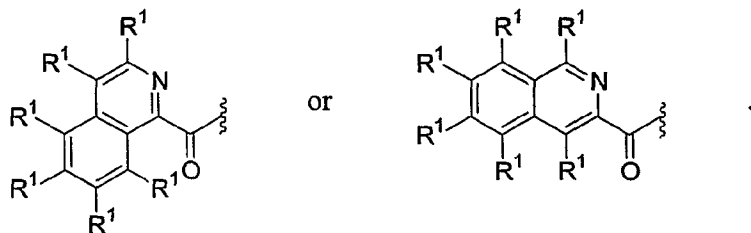


the isoquinoline group has bonded to each non-bridgehead carbon either a group R^1 or the group $-\text{C}(=\text{O})-(\text{NH}-\text{Y}-\text{CO})_m-\text{Z}(\text{R}^2)_n$. Preferably, the latter group is bonded to either the isoquinoline C1 or C3. That is, the residue

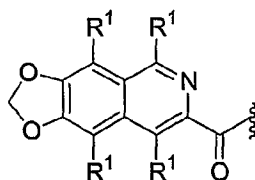


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preferably is

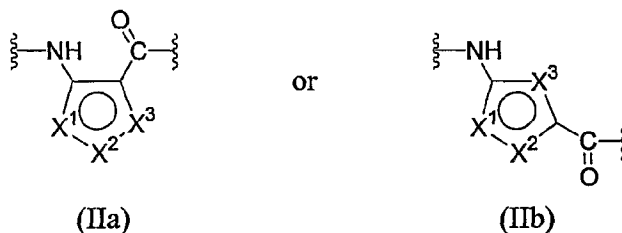


[43] Also, two moieties R^1 may be connected to form a cyclic structure, a preferred example of such embodiment being:



5 Preferably, each of the R^1 moieties in the immediately preceding three formulae is H.

[44] Moieties M^1 , described by formulae IIa and IIb

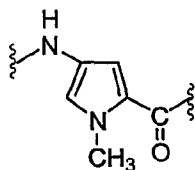


provide additional heteroaromatic polyamide building blocks. Moieties M^1 are 5-membered ring heteroaromatic moieties, the selection of X^1 , X^2 , and X^3 determining the type of heteroaromatic ring. Exemplary heteroaromatic rings include imidazole, pyrrole, 10 pyrazole, furan, isothiazole, oxazole, isoxazole, thiazole, furazan, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadiazole, 1,2,3-triazole, 1,2,4-triazole, 1,3,4-oxadiazole, 1,2,4-oxadiazole, and thiophene. Preferably, at least one moiety Y is a moiety M^1 .

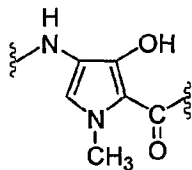
15 [45] The circle in the five-membered rings of formulae IIa and IIb is meant to indicate the presence of two double bonds, which, in some embodiments, can move within the ring.

[46] Preferred moieties M^1 are IIc (hereinafter "Py"), formally derived from 1-methyl-4-aminopyrrole-2-carboxylic acid, IId (hereinafter "Hp"), formally derived from 1-

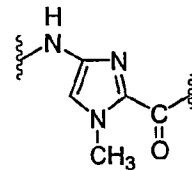
methyl-3-hydroxy-4-aminopyrrole-2-carboxylic acid, and Ile (hereinafter "Im"), formally derived from 1-methyl-4-aminoimidazole-2 carboxylic acid:



(IIc)



(IIId)



(IIe)

[47] It has been shown by Dervan and co-workers (see, e.g., Dervan, US 6,143,901 (2000); Baird et al., WO 98/37066 (1998); White et al., Nature **391**, 468 (1998); White et al., Chem. Biol. 1997, **4**, 569) that, in a 2:1 binding mode to dsDNA, moieties Py, Im, and Hp moieties can be used to recognize specific dsDNA base pairs, giving rise to a set of "pairing rules" correlating heteroaromatic moiety pairs and DNA base pairs. These pairing rules are summarized below:

10	<u>Heteroaromatic Pair</u>	<u>dsDNA Base Pair(s) Recognized</u>
	Im/Py	G/C
	Py/Im	C/G
	Py/Py	A/T, T/A (degenerate)
	Hp/Py	T/A
15	Py/Hp	A/T

Such recognition can lead to sequence-specific dsDNA binding, enabling the design of compounds (I) that target predetermined DNA base pair sequences, for example, a specific promoter site or a sequence characteristic of a gene.

[48] Optionally, compound (I) can include one or more moieties M²



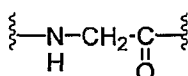
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A moiety M² can function as a "spacer" for adjusting the positioning of the heteroaromatic moieties M¹ or M⁴ relative to the dsDNA base pairs at the binding site. As a compound (I) binds in the minor groove, the alignment of heteroaromatic moieties M¹ and M⁴ with the DNA base pairs with which they to interact of optimal binding or sequence recognition may drift as the number of heteroaromatic moieties M¹ and M⁴ increases. Alternatively, incorporation of a moiety M² adds flexibility to compound (I),

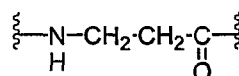
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allowing its curvature to more accurately match that of the minor groove. The incorporation of one or more flexible moieties M^2 relaxes the curvature of the compound backbone, permitting larger compounds (I) to bind to longer sequences of DNA. In some preferred embodiments a moiety M^2 is present for every 4 to 5 heteroaromatic moieties M^1 or M^4 , more preferably interrupting long sequences of M^1 and/or M^4 groups.

[49] Preferred moieties M^2 are those corresponding to glycine ($x = 0$ in formula III, depicted as IIIa below) and β -alanine ($n = 1$ and $R^{15} = H$ in formula III; depicted as IIIb below, hereinafter " β "), with the latter being especially preferred.



(IIIa)



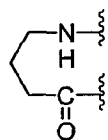
(IIIb)

[50] Moieties M^2 in which $x = 1$ and $R^{15} = OH, NH_2$, or F can be used to alter the binding affinity and specificity (relative to β -alanine), as disclosed in Floreancig et al., *J. Am. Chem. Soc.*, 2000, **122**, 6342; the disclosure of which is incorporated herein by reference.

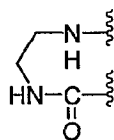
[51] When present in compound (I), optional moieties M^3 (formula IV)



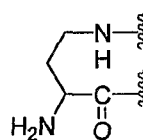
have a group L providing a spacer of 3 to 4 atoms between $-\text{NH}-$ and $-\text{C}(=\text{O})-$ and can be used to introduce a hairpin turn into compound (I). See Mrksich et al., *J. Am. Chem. Soc.* 1994, **116**, 7983. Exemplary moieties M^3 include:



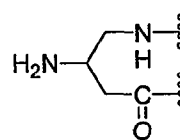
(IVa)



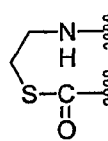
(IVb)



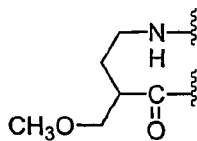
(IVc)



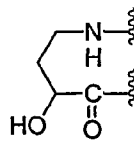
(IVd)



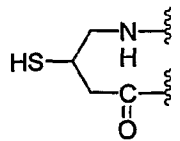
(IVe)



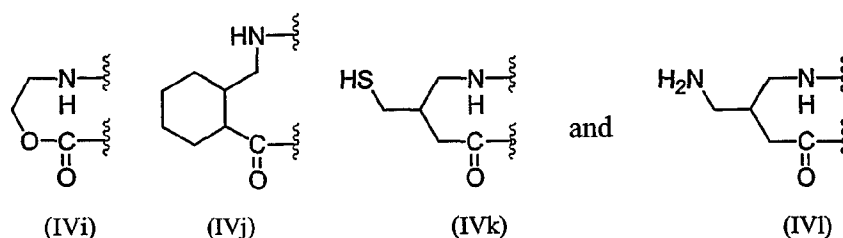
(IVf)



(IVg)

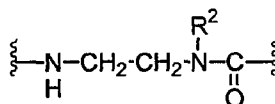


(IVh)



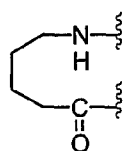
[52] Moieties IVa (hereinafter “ γ ”), corresponding to γ -aminobutyric acid, and IVc, corresponding to 2,4-diaminobutyric acid, are preferred. Selecting one enantiomer or the other of moieties M^3 that are chiral allows stereochemical control of the binding of polyamides to the minor groove, for example as disclosed in Baird et al., WO 98/45284 (1998) in respect of R-2,4-diaminobutyric acid and S-2,4-diaminobutyric acid (corresponding to R-IVc and S-IVc, respectively).

[53] Yet another class of moieties M^3 is represented by the formula



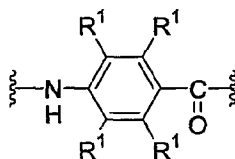
where R^2 is as previously defined.

[54] While the group L preferably provides a 3-atom separation between the ---NH--- and the ---(C=O)--- , a 4-atom separation is also permissible, as illustrated by a 5-aminovaleric acid residue (i.e., L equals $\text{---(CH}_2\text{)}_4\text{---}$):

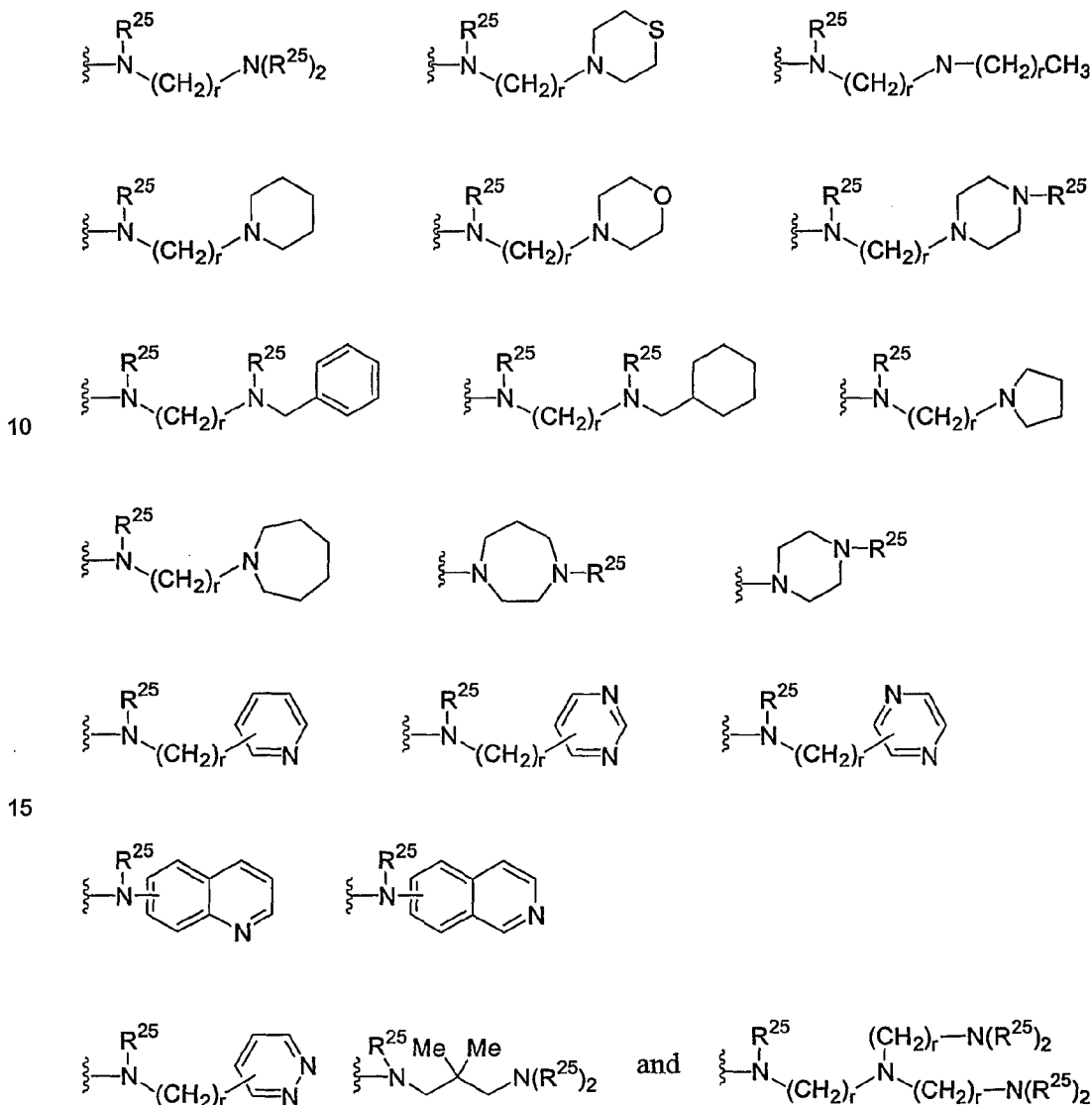


[55] L can have pendant groups, which serve to enhance solubility or function as attachment points for other groups (e.g., IVc, IVd, IVg, IVh, IVk, IVl). The 3 to 4 atoms can be part of a larger group, which provides conformational rigidity (e.g., IVj). The 3 to 4 atoms can comprise carbon atoms only or it can include heteroatoms (e.g., IVb, IVe, IVi).

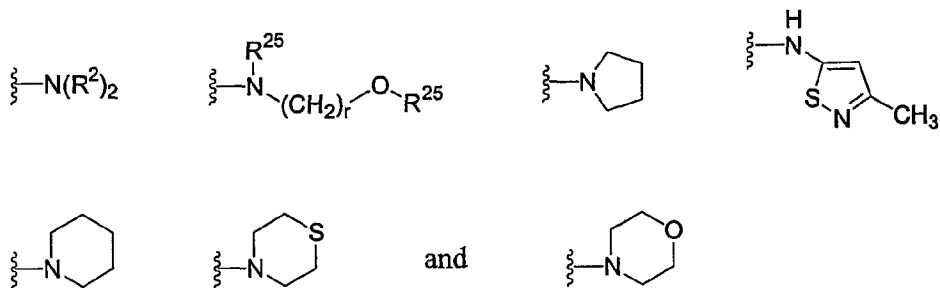
[56] Moieties M^4 are used to introduce a benzamide unit into compound (I). Preferably, the benzamide unit is *para*-oriented, as in



[57] The group $Z(R^2)_n$ can be viewed as a terminal group, located at the C-terminus and forming an amide or ester cap there. In the case of $Z = N$, the two groups R^2 can be linked to each other to form a cyclic structure. A group $Z(R^2)_n$ can contain a basic group (as defined hereinbelow). Examples of suitable groups containing a basic group include:



[58] Examples of suitable groups $Z(R^2)_n$ not containing a basic group include:



[59] In the foregoing formulae, r is an integer ranging from 2 to 8, inclusive (preferably 2 to 6), and each R^{25} is independently H, CH_3 , CH_2CH_3 , $CH_2CH_2CH_3$, or $CH(CH_3)_2$.

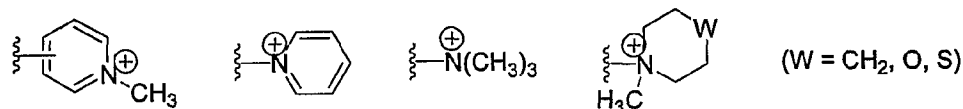
[60] The classification of the 5-amino-2-methylisothiazole group as a “nonbasic” $Z(R^2)_n$ group is somewhat arbitrary, as its pK_b is marginal, normally around 12-13 (i.e., pK_a 1-2) and depending on the molecular structure of the entire compound, it may qualify or not as a basic group as such is defined herein. Preferably, where a 5-amino-2-methylisothiazole is present, the compound has a basic group elsewhere in the molecule, for example pendant from a moiety M^1 or M^4 , as exemplified by compounds Ib-39 and Ib-40, *infra*.

[61] The preceding illustrative formulae of basic and nonbasic groups $Z(R^2)_n$ have been drawn with Z as N and n as 2 for convenience. Those skilled in the art will appreciate that in these formulae the residue NR^{25} can be replaced with O to give the corresponding groups $Z(R^2)_n$ in which Z is O and n is 1. Where Z is O, preferably the adjacent moiety Y is Py.

[62] As used herein with reference to groups R^1 and R^2 , “substituted or unsubstituted (C_1 - C_{12})alkyl group, or a substituted or unsubstituted (C_1 - C_{12})heteroalkyl group” includes not only conventional alkyl or cycloalkyl groups such as methyl, ethyl, propyl, isopropyl, butyl, s-butyl, isobutyl, t-butyl, cyclopentyl, cyclohexyl, and pentyl, but also unsaturated C_1 to C_{12} groups, having for example aromatic, alkenyl, or alkynyl groups (e.g., phenyl, benzyl, vinyl, cyclohexenyl, etc.). One or more backbone carbons can be replaced by heteroatoms. There may be present functionalities such as hydroxy; oxo ($=O$); primary, secondary, or tertiary amine (e.g., $-NH_2$, $-NH(CH_3)$, $-N(CH_3)_2$); quaternary ammonium (e.g., $-N(CH_3)_3^+$); alkoxy (e.g., methoxy, ethoxy); acyl (e.g., $-C(=O)CH_3$); amide (e.g., $-NHC(=O)CH_3$); thiol; thioether (e.g., $-SCH_3$); sulfoxide; sulfonamide (e.g., $-SO_2NHCH_3$); halogen (e.g., F, Cl); nitro; and the like. Exemplary specific R^1 and R^2 groups include methyl, trifluoromethyl, ethyl, acetyl, methoxy, methoxyethyl,

ethoxyethyl, aminoethyl, hydroxyethyl, propyl, hydroxypropyl, cyclopropyl, isopropyl, 3-(dimethylamino)propyl, butyl, s-butyl, isobutyl, t-butyl, pentyl, cyclopentyl, vinyl, allyl, ethynyl, propynyl, and the like.

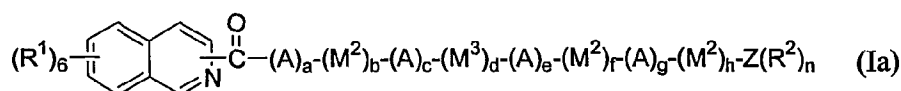
[63] Compound (I) has a basic group having a pK_b of 12 or less or a quaternized nitrogen group. (Or, stated conversely, the conjugate acid of the basic group has a pK_a greater than 2 ($pK_a = 14 - pK_b$).) Preferably, the pK_b is less than 10, more preferably less than 5. A pK_b of less than 12 ensures that compound (I) is protonated under the conditions in which it interacts with a nucleic acid. Preferably the basic group is a nitrogenous group, for example an amine, an amidine, a guanidine, a pyridine, a pyridazine, a pyrazine, a pyrimidine, an imidazole, or an aniline. Primary, secondary, or tertiary aliphatic amines, are preferred. Exemplary quaternized nitrogen groups include alkyl pyridinium and tetraalkyl ammonium groups such as:



[64] A basic/quaternized nitrogen group may improve cell transport properties, enabling the compounds of this invention to be transported across cellular and nuclear membranes and to reach dsDNA in the nucleus. See Rothbard et al., WO 98/52614 (1998), which discloses that guanidine or amidino side chain moieties enhance transport across biological membranes. Another possible benefit is enhancement of the binding affinity to the nucleic acid, perhaps via ionic interactions with backbone phosphate groups. See Baird and Dervan, WO 98/37087 (1998) and Bruice et al., US 5,698,674 (1997). Lastly, the protonated basic or quaternized nitrogen group enhances the solubility of compounds (I).

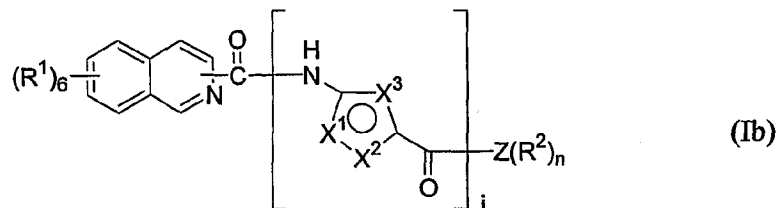
[65] Preferably, the basic or quaternized nitrogen group is present within the C-terminal group $Z(R^2)_n$, but it may be present elsewhere in the molecule, for example as part of a group R^1 or R^2 in M^1 or M^4 . Or, multiple basic or quaternized nitrogen groups may be present, at different locations on compound (I).

[66] In a preferred embodiment, compound (I) is according to formula (Ia):

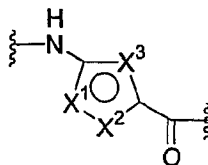


wherein M^2 , M^3 , R^1 , R^2 , Z and n have the same meanings as previously assigned; each A is independently M^1 or M^4 ; each of a , c , e , g and h is an integer independently from 0 to 5, inclusive; and each of b , d , and f is independently 0 or 1. The sum of a , c , e , and g is preferably at least 2, more preferably at least 3.

- 5 [67] In another preferred embodiment, compound (I) is according to formula (Ib):

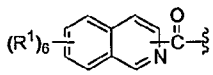
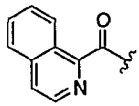
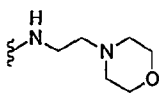
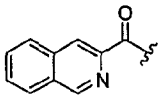
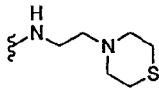
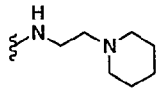
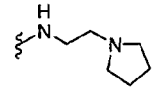
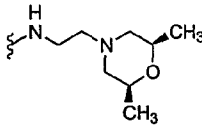
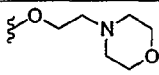
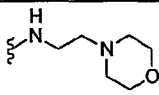
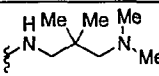
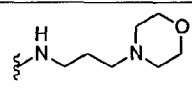
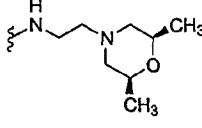
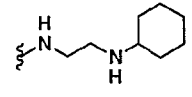


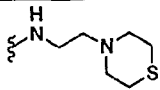
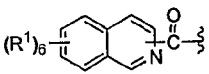
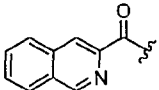
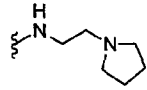
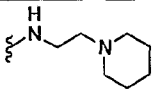
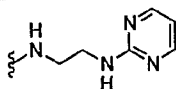
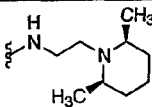
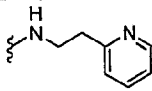
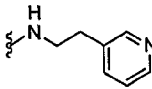
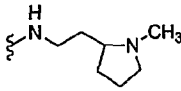
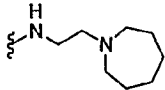
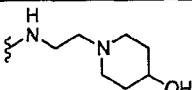
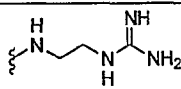
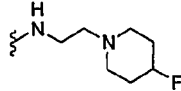
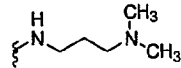
wherein X^1 , X^2 , X^3 , R^1 , R^2 , Z , and n have the meanings previously assigned and i is an integer between 2 and 4, inclusive. In one subset of compounds according to formula (Ib), each of the moieties

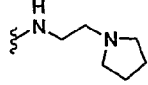
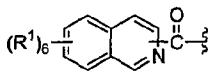
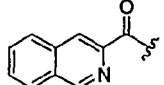
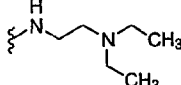
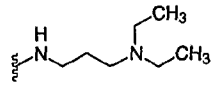
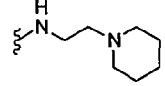
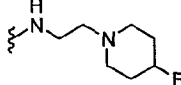
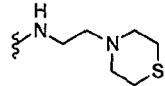
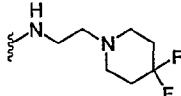
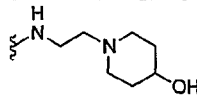
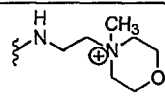
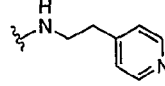
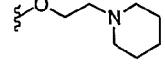
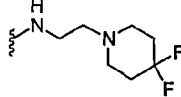
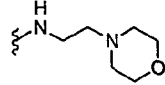


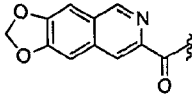
10

is Py, as exemplified by the compounds in Table A:

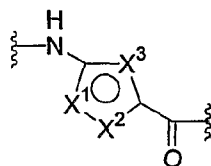
Table A — Illustrative Compounds (Ib)			
Compound Ref.		i	$\text{---Z(R}^2\text{)}_n$
Ib-1		3	
Ib-2		3	Same
Ib-3	Same	3	
Ib-4	Same	3	
Ib-5	Same	3	
Ib-6	Same	3	
Ib-7	Same	3	
Ib-8	Same	4	
Ib-9	Same	3	
Ib-10	Same	3	
Ib-11	Same	2	
Ib-12	Same	3	

Ib-13	Same	2	
Compound Ref.		i	$\text{---Z(R}^2\text{)}_n$
Ib-14		2	
Ib-15	Same	2	
Ib-16	Same	3	
Ib-17	Same	3	
Ib-18	Same	3	
Ib-19	Same	3	
Ib-20	Same	3	
Ib-21	Same	3	
Ib-22	Same	3	
Ib-23	Same	3	
Ib-24	Same	3	
Ib-25	Same	4	

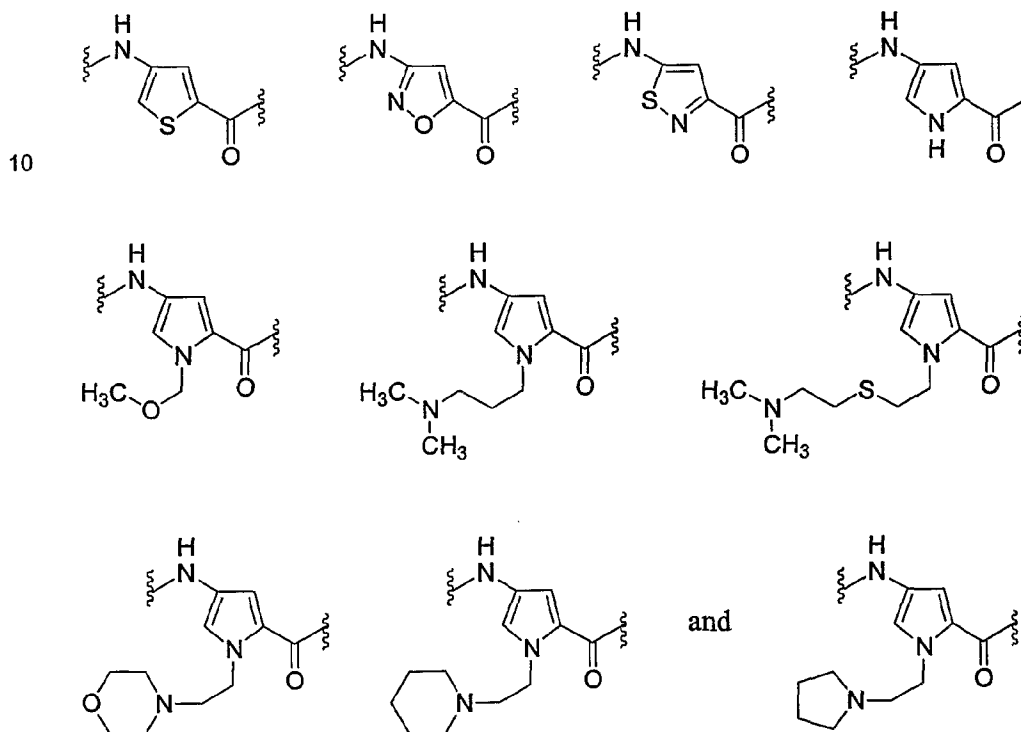
Ib-26	Same	4	
Compound Ref.		i	$\text{Z}(\text{R}^2)_n$
Ib-27		4	
Ib-28	Same	4	
Ib-29	Same	4	
Ib-30	Same	4	
Ib-31	Same	4	
Ib-32	Same	4	
Ib-33	Same	4	
Ib-34	Same	3	
Ib-35	Same	3	
Ib-36	Same	3	
Ib-37	Same	3	
Ib-38	Same	3	

Ib-39		2	Same
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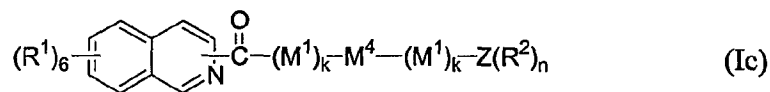
However, the moieties



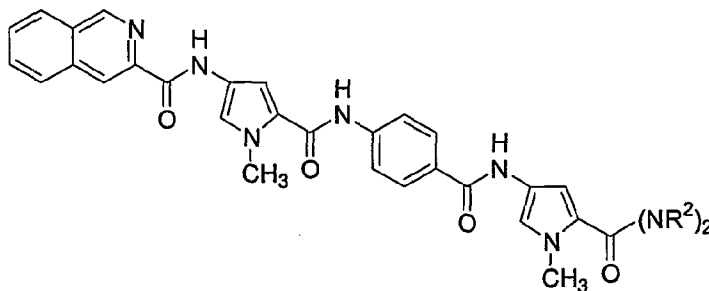
need not all be Py. They can be other 5-member ring heterocycles, as illustrated by
 5 compounds **Ib-50** through **Ib-93** shown in Figs. 1a through 1f. As illustrated by
 compounds such as **Ib-55** to **Ib-57**, **Ib-63** to **Ib-66**, **Ib-68**, and **Ib-73** to **Ib-74**, compounds
Ib can have basic or quaternized nitrogen groups pendant from a 5-member heterocycle,
 instead or in addition to a basic/quaternized nitrogen group in the C-terminal $Z(R^2)_n$.
 Preferred non-Py 5-member ring heterocycles are selected from the group consisting of



15 [68] In yet another preferred embodiment, compounds (I) of this invention are
 according to formula (Ic):

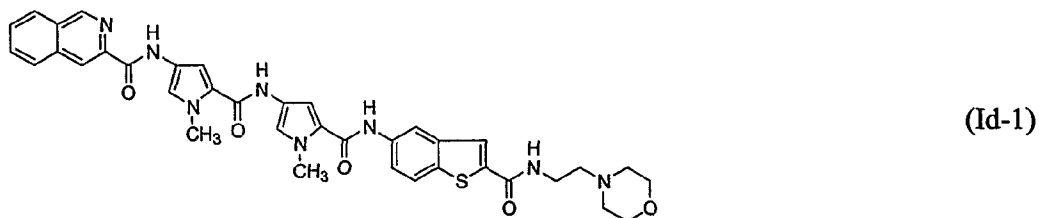


wherein M^4 , R^1 , R^2 , Z , and n are as previously defined and each k is independently an integer from 0 to 4, inclusive. A preferred subclass of compounds (Ic) is of the formula



5 [69] Illustrative compounds (Ic) are shown in Figs. 2a through 2c.

[70] Compounds of formula (I) can have a 6,5-condensed heteroaromatic ring system, either individually or linked to another aromatic or heteroaromatic ring system, as illustrated by compound **Id-1**.



10 [71] Compounds (I) can be conjugated or linked to another nucleic acid binding compound. The conjugated nucleic acid binding compounds can be two identical or different compounds (I), or one compound (I) and a different class of nucleic acid binder, for example an intercalator, a triple helix former, a binder to the phosphate backbone, a major groove binder, another type of minor groove binder, and the like. A preferred site
15 for forming the conjugating link is an amino, hydroxy, or thiol functionality in a group L in moiety M^2 , which can be acylated or alkylated. The preparation of tandem linked nucleic acid binding polyamides in this manner is disclosed in Baird et al., WO 98/45284 (1998), the disclosure of which is incorporated herein by reference.

[72] Compounds (I) also can be conjugated to other moieties, such as, peptides,
20 proteins, transport agents, fluorophores or other reporter groups, and the like.

[73] Compounds (I) preferably bind to dsDNA with high affinity, meaning an equilibrium association constant of at least 10^3 M^{-1} , more preferably at least 10^6 M^{-1} , and most preferably at least 10^9 M^{-1} . The measurement of binding affinities by quantitative DNase I footprinting is disclosed in Dervan, WO 98/50582 (1998), and Trauger et al.,
5 *Nature* **382**, 559 (8 Aug. 1996); the disclosures of which are incorporated herein by reference.

[74] Compounds of this invention can be used to form complexes with dsDNA, for the purpose of recognizing and/or isolating dsDNA strands containing particular base-pair sequences, for example for analytical or diagnostic purposes. Thus, in another aspect of
10 this invention there is provided a complex between dsDNA and compound of this invention. In cellular systems or in living organisms, they can modulate the expression of a gene by binding to the gene or a promoter or repressor region thereof. Such modulation may be useful for therapeutic or research purposes.

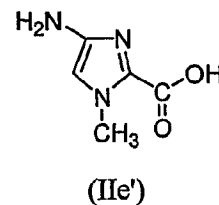
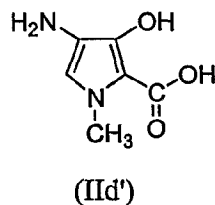
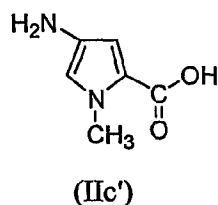
[75] Additionally, compounds of this invention have been found to have anti-bacterial
15 and/or antifungal properties and therefore may be used for combating (i.e., preventing and/or treating) infections in eukaryotic organisms. Other pathogens against which compounds of this invention can be useful include protozoa and viruses. For human anti-infective applications, an effective amount of a compound of this invention is used, optionally in combination with a pharmaceutically acceptable carrier. The composition
20 may be dry, or it may be a solution. Treatment may be reactive, for combating an existing infection, or prophylactic, for preventing infection in an organism susceptible to infection. Preferably, compounds of this invention are used to treat infections by drug-resistant strains of bacteria, for example MRSA (methicillin resistant *S. aureus*), MRSE (methicillin resistant *S. epidermidis*), PRSP (penicillin resistant *S. pneumoniae*) or VRE
25 (vancomycin resistant *Enterococci*). By "drug-resistant" it is meant that the bacteria are resistant to treatment with conventional antibiotics.

[76] Host organisms that can be treated include eukaryotic organisms, in particular plants and animals. The plant may be an agriculturally important crop, such as wheat, rice, corn, soybean, sorghum, and alfalfa. Animals of interest include mammals such as
30 bovines, canines, equines, felines, ovines, porcines, and primates (including humans). Thusly, in another aspect of this invention, there is provided a method for treating a bacterial infection — particularly an infection by Gram-positive bacteria — comprising

administering to a patient in need of such treatment an effective amount of compound (I). Compounds of this invention can be used in the preparation of a medicament for treating a bacterial infection in a mammal. The compounds may be administered orally, topically, or parenterally (e.g., intravenously, subcutaneously, intraperitoneally, transdermally).

5 [77] While not wishing to be bound by any particular theory, it is believed that the compounds of this invention derive their biological activity from their ability to bind to dsDNA.

[78] Compounds I can be synthesized by solid phase techniques from the corresponding amino acids or their derivatives, for instance IIc', IId', and IIe' for the
10 synthesis of the Py, Hp, and Im building blocks, respectively.



In solid phase synthesis, a polyamide is synthesized on a resin such as Boc-glycine-PAM-resin or Boc-β-alanine-PAM-resin, with moieties Y being added in series of steps involving amino-protected and carboxy-activated monomers, as taught in Dervan et al.,
15 US 6,090,947 (2000) (the “947 patent”); Baird et al., WO 98/37066 (1998); Baird et al., WO 98/37067 (1998); and Dervan et al., WO 98/49142 (1998); Baird et al., U.S. Application Ser. No. 10/132,887, filed April 24, 2002; McMinn, U.S. Provisional Application Ser. No. 60/298,206, filed June 13, 2001 (the “206 application”); Ge et al., WO 01/74898 (2001) (the “898 application”); Kelly et al., *Proc. Nat’l Acad. Sci. USA*,
20 July 1996, 93, 6981 (“Kelly”); and Wade et al., *J. Am. Chem. Soc.*, 1992, 114, 8783 (“Wade”); the disclosures of which are incorporated herein by reference.

[79] The practice of this invention may be further understood by reference to the following examples, which are provided by way of illustration and not of limitation.

Synthesis of Compounds

General

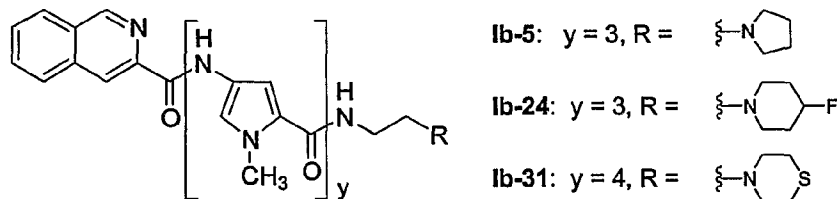
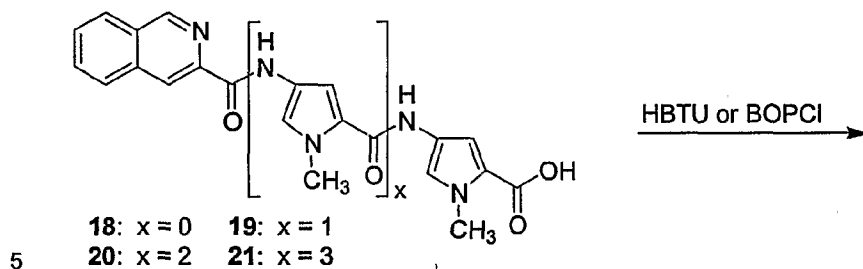
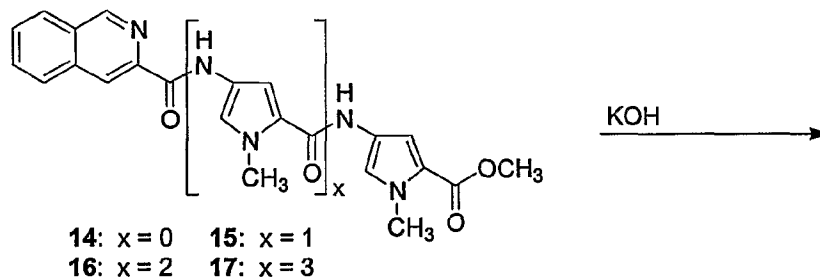
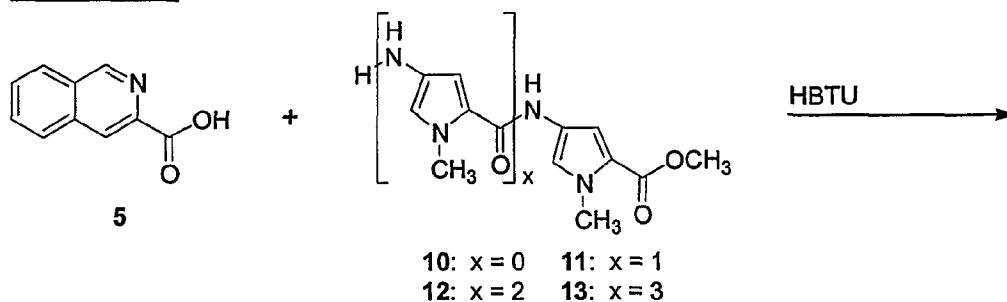
[80] Typically, the structures of compounds were confirmed by ¹H-NMR and/or mass spectrometry. Where a parenthetical remark such as “¹H-NMR” or “mass spectrum” follows a reference to a compound without any elaboration, it means that such spectrum was taken, was consistent with the assigned structure, and did not indicate the presence of significant impurities.

[81] Abbreviations in common usage are employed for various solvents, catalysts and reagents, including: HBTU for 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexa-for dicyclohexyl-carbodiimide; BOPCl for bis(2-oxo-3-oxa-zolidinyl)-phosphinic chloride; HATU for O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexa-fluorophosphate; and MOMCl for methoxymethyl chloride.

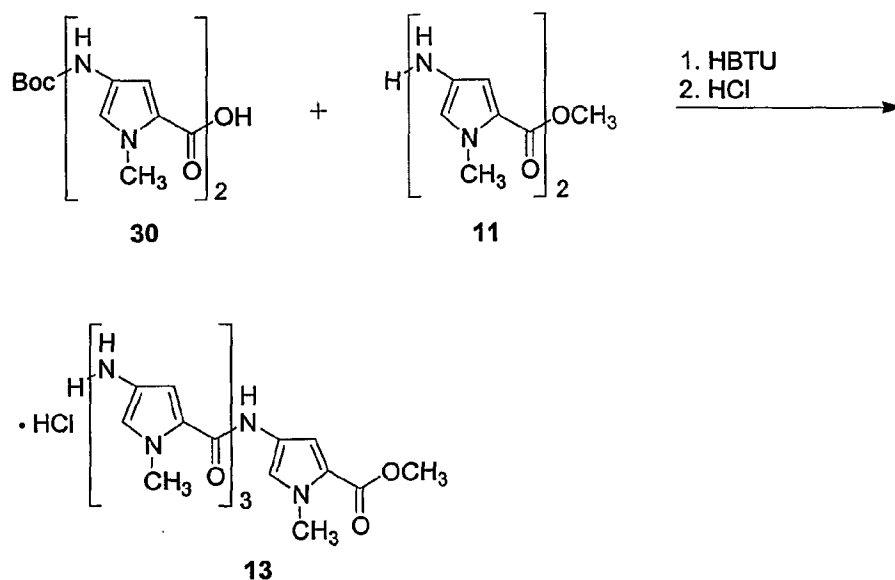
[82] The skilled artisan will understand: (a) that an intermediate described in the context of the synthesis of a particular compound of this invention can also be used to make other compounds of this invention, *mutatis mutandis*; (b) that in certain experimental sections only the preparation of an intermediate compound is described, because its incorporation into a final compound of this invention straightforwardly follows synthetic methodology described herein; and (c) that, for some routine reactions (HBTU-mediated couplings, hydrogenations, saponifications, etc.) that recur herein, detailed reaction and work-up conditions sometimes are not provided after the first few recurrences in the interest of brevity and that the conditions described elsewhere in this application are adaptable to the instance at hand without undue experimentation.

Example A

[83] Scheme A-1 describes the synthesis of intermediates containing an isoquinoline carboxamide moiety having adjacent thereto one to four N-methylpyrrole carboxamide (“Py”) units. The syntheses of acids 19 and 21 are described in detail; acids 18 and 20 can be made by analogy. The preparations of compounds Ib-5, Ib-24 and Ib-31 are given as representative examples.

Scheme A-1

[84] *Starting materials.* Starting materials 10-13 are known compounds (CAS Reg. Nos. 180258-45-1, 126092-99-7, 162085-96-3, and 295805-47-9, respectively). Tetramer 13 was prepared by coupling Boc-protected acid 30 and ester 11 with HBTU followed by de-protection with HCl, as shown in Scheme A-2. (Acid 30 can be prepared from ester 11 by Boc-protecting the amino group and saponifying the ester group.)

Scheme A-2

- [85] **Compounds 15 and 19.** A mixture of isoquinoline-3-carboxylic acid **5** (3.32 g, 1.2 equiv.) and HBTU (6.91 g, 1.14 equiv.) in DMF (30 mL) and DIEA (6 mL) was stirred at room temperature ("RT") for 45 min and added to a solution of amino ester **11** (5.00 g, 1.0 equiv.) in DMF (30 mL) and DIEA (6 mL). The mixture was stirred for 20 hr at RT and poured into ice-water (*ca.* 700 mL). The resulting precipitate was collected by filtration to yield crude compound **15**. The precipitate was suspended in EtOH (100 mL) and H₂O (100 mL). The mixture was treated with KOH (5 g), stirred at 70 to 80°C for 17 hr, diluted with H₂O (*ca.* 600 mL) and washed with ethyl acetate ("AcOEt") (1x). The aqueous layer was acidified to pH 3 with 1M aqueous HCl and the resulting solids collected by filtration and dried *in vacuo* to yield acid **19** (2.8 g, 42% yield over two steps, ¹H-NMR).
- [86] **Compounds 17 and 21.** A mixture of isoquinoline-3-carboxylic acid **5** (4.47 g, 1.15 equiv.) and HBTU (9.36 g, 1.1 equiv.) in NMP (50 mL) and DIEA (10 mL) was stirred for 45 min at RT and added to a solution of amino ester **13** (12.51 g, 1.0 equiv.) in NMP (10 mL) and DIEA (2 mL). The mixture was stirred for 16 hr at RT and added dropwise to 10% aqueous K₂CO₃ (800 mL). The resulting precipitate of compound **17** (¹H-NMR) was collected by filtration. The filter cake was suspended in MeOH (200 mL) and 2M aqueous NaOH (200 mL) and stirred for 16 hr at 60°C. The mixture was diluted with H₂O (500 mL) and acidified to pH 2 with *ca.* 4M aqueous HCl. The solids were

collected by filtration and dried in vacuo to yield the carboxylic acid **21** (14.15 g, 89% over two steps).

[87] Compound Ib-5. A mixture of acid **20** (140 mg, 1.0 equiv.) and HBTU (118 mg, 1.2 equiv.) in NMP (1 mL) and DIEA (0.3 mL) was stirred at 37°C for 1 hr and treated
5 with 1-(2-aminoethyl)pyrrolidine (0.4 mL). The mixture was stirred for 16 hr (37 °C),
diluted with 50% aqueous AcOH (10 mL), washed with AcOEt (1x) and purified by
HPLC to yield compound **Ib-5** (¹H-NMR; mass spectrum).

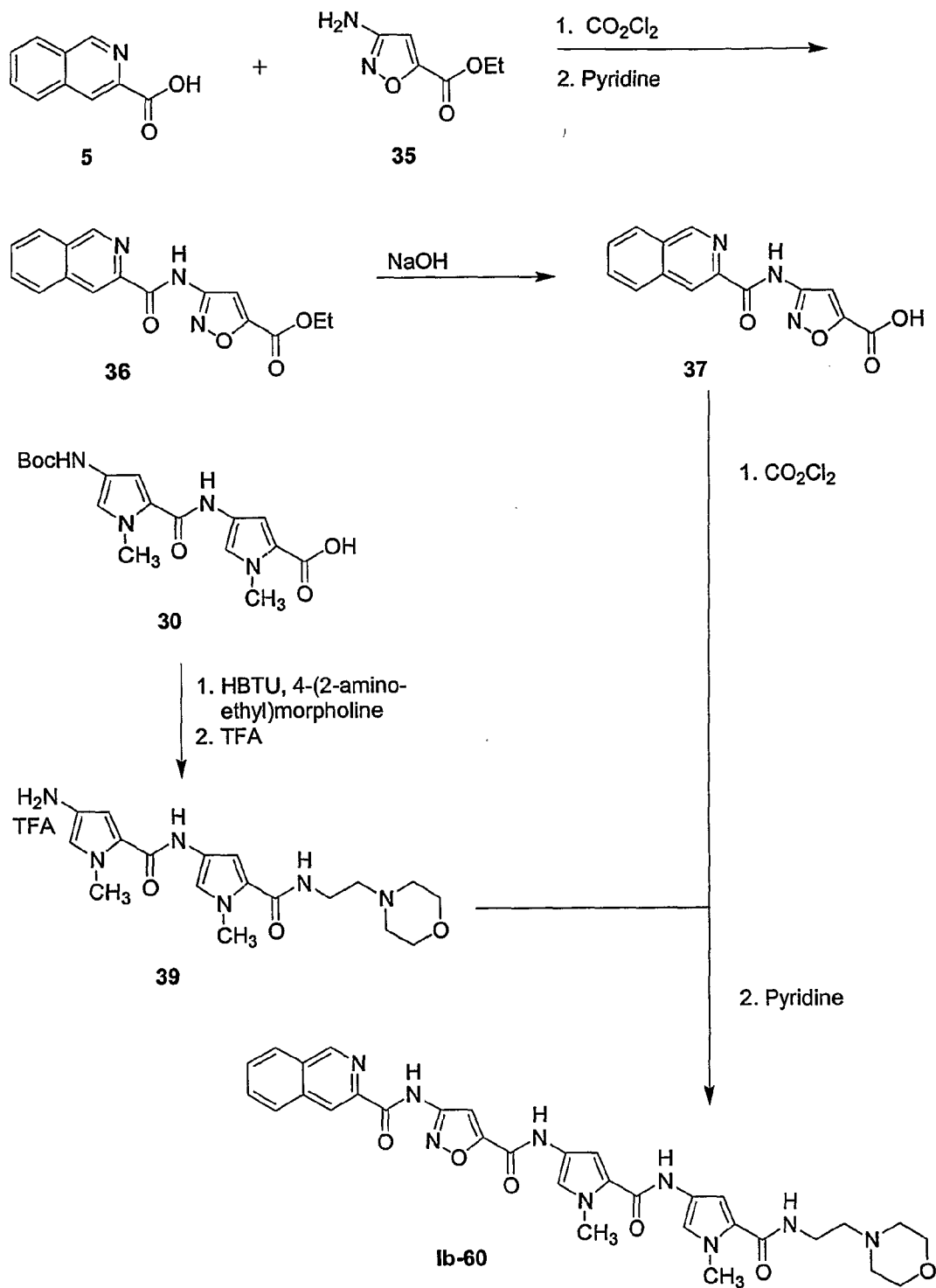
[88] Compound Ib-24. A mixture of acid **20** (100 mg, 1.0 equiv.) and BOPCl (58.2 mg, 1.2 equiv.) in NMP (1 mL) and DIEA (0.1 mL) was stirred at 37°C for 45 min and treated
10 with 1-(2-aminoethyl)-4-fluoropiperidine (0.08 mL, *ca.* 3 equiv.). The mixture was stirred
for 16 hr (37 °C), diluted with 50% aqueous AcOH (10 mL) and purified by HPLC to
yield compound **Ib-24** (¹H-NMR; mass spectrum).

[89] Compound Ib-31. A mixture of acid **21** (90 mg, 1.0 equiv.) and BOPCl (41.5 mg, 1.2 equiv.) in NMP (1 mL) and DIEA (0.1 mL) was stirred at 37°C for 45 min and treated
15 with 4-(2-aminoethyl)thiomorpholine (0.1 mL, *ca.* 5 equiv.). The mixture was stirred for
16 hr (37 °C), diluted with 50% aqueous AcOH (10 mL) and purified by HPLC to yield
compound **Ib-31** (¹H-NMR; mass spectrum).

Example B

[90] Scheme B-1 illustrates the synthesis of compounds having a heteroaromatic
20 carboxamide residue other than Py — in this instance an isoxazole carboxamide residue
— with compound **Ib-60** as a prototype.

Scheme B-1



[91] Ester amide 36. Oxalyl chloride (1.67 mL, 19.19 mmol) was added dropwise to a suspension of isoquinoline-3-carboxylic acid **5** (332.3 mg, 1.92 mmol) in THF (2 mL)

and the reaction mixture was heated at reflux (85 °C oil bath) for 3 hr. All volatile components were removed *in vacuo*. The resulting solid (presumed acid chloride) was dissolved in NMP (1 mL) and pyridine (1 mL), and ethyl 3-aminoisoxazole-5-carboxylate 35 (Lepage *et al.*, FR 2,750,425 (1998), 300mg, 1.92 mmol) was added. The reaction mixture was stirred at RT for 3 hr and then added drop-wise to a rapidly stirred solution of ice-cold water (2 mL), causing precipitation of ester-amide 36 as a white solid (300 mg, 50%, ¹H-NMR), which was collected by filtration and dried by lyophilisation.

[92] Acid 37. Aqueous sodium hydroxide (1N, 2 mL, 2 mmol) was added to a solution of ester-amide 36 (250 mg, 0.803 mmol) in ethanol (2 mL). The reaction mixture was stirred for 30 minutes at RT, at which point TLC analysis indicated complete consumption of starting material. The mixture was then acidified to pH 2-3 with 2N aq. HCl, causing precipitation of the product acid 37 as a white solid (245 mg, quantitative yield, ¹H-NMR), which was collected by filtration and dried by lyophilisation.

[93] Amine 39. Carboxylic acid 30 (Scheme A-2) was converted to amide-amine 39 by activation with HBTU (0.95 eq.) in DMF/TEA at RT for 45 min, followed by addition of 4-(2-aminoethyl)morpholine (1.2 eq.) and reaction at 37 °C overnight. Volatiles were removed *in vacuo*, and TFA was added. The reaction mixture was stirred at RT for 3 hr. Work-up yielded amide-amine 39 as the trifluoroacetate salt.

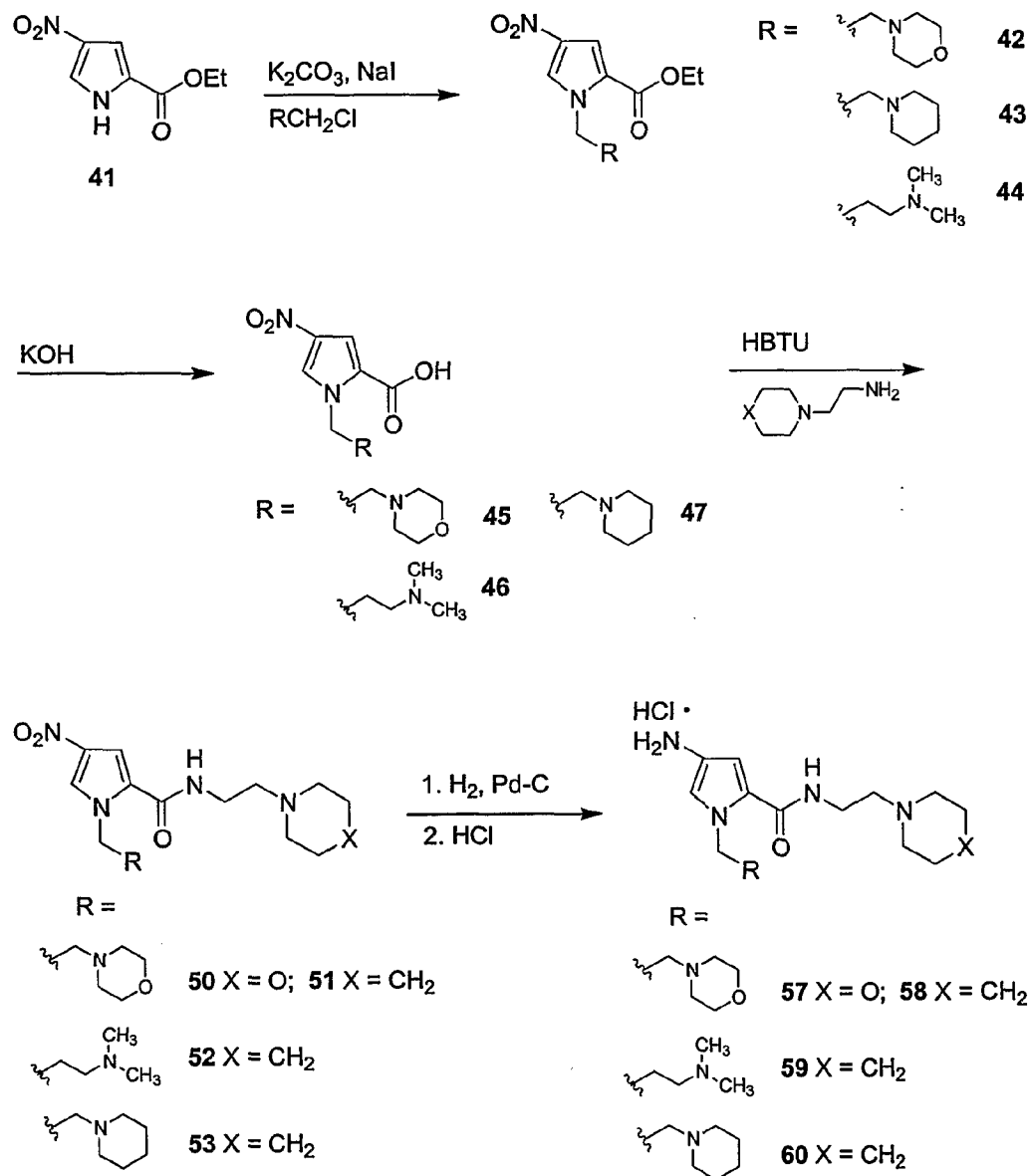
[94] Compound Ib-60. Oxalyl chloride (0.22 mL, 2.54 mmol) was added drop-wise to a suspension of acid 37 (72 mg, 0.254 mmol) in THF (1 mL) and the reaction heated at reflux (oil bath 85 °C) for 3 hours. All volatile components were removed *in vacuo*. The resulting solid (presumed acid chloride) was dissolved in NMP (0.5 mL) and pyridine (0.5 mL). A solution of amine 39 (105 mg, 0.254 mmol) in NMP (1 mL) and DIEA (0.5 mL) was added. The reaction mixture stirred at 60 °C for 12 hours, diluted with 50% acetic acid solution, and directly purified by HPLC to give the desired compound **Ib-60** (25 mg, 16%, ¹H-NMR).

Example C

[95] This example describes the synthesis of intermediates for the preparation of compounds of this invention having pyrrole carboxamide units in which the pyrrole nitrogen is substituted with a substituent other than methyl, as in the instance of

compounds **Ib-55** to **Ib-57**, **Ib-63** to **Ib-66**, and **Ib-68**. The synthetic methodology is shown in Scheme C-1.

Scheme C-1



[96] Compounds 42-44. The synthesis of compounds **42-44** is illustrated with compound **42**, the other compounds being analogously synthesizable. A mixture of ethyl 4-nitropyrrole-2-carboxylate **41** (20.00 g, 1.0 equiv.), 4-(2-chloroethyl)-morpholine hydrochloride (28.28 g, 1.4 equiv.), NaI (16.28 g, 1.0 equiv.) and K_2CO_3 (28.78 g, 1.92 equiv.) in DMF (200 mL) was stirred at 60°C for 10.5 hr and poured into a mixture of

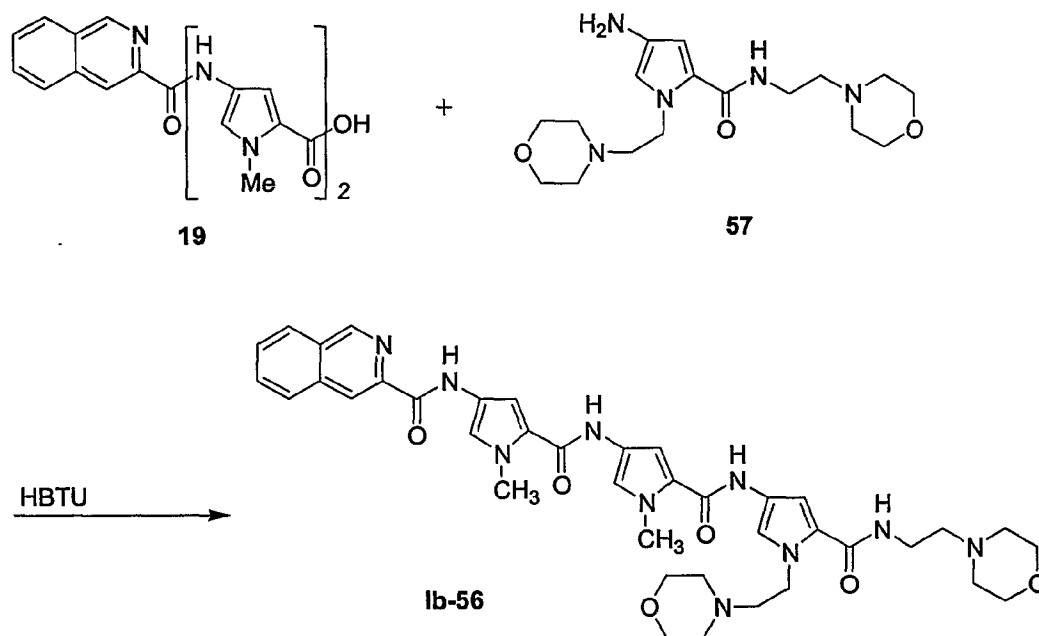
H₂O and saturated aq. K₂CO₃ (550/50 mL). The resulting solution was extracted with AcOEt (4x, each 200 mL). The combined organic layers were dried (MgSO₄) and evaporated to give compound **42** as pale yellow crystals (31.4 g, 97%, ¹H-NMR).

5 [97] Compounds 45. A suspension of ester **42** (31.4 g, 1.0 equiv.) and KOH (8.13 g, 2 equiv.) in EtOH (100 mL) and H₂O (100 mL) was stirred at RT for 16 hr (complete dissolution after 1 hr). The mixture was acidified with 1M aq. HCl to pH 3.0 and the resulting precipitate was collected by filtration and dried *in vacuo* to give compound **45** as a white solid (23.0 g, 81%, ¹H-NMR). Compounds **46** and **47** were analogously synthesized, except that workup was by neutralization to pH 7 with 1M HCl and
10 evaporation.

[98] Compounds 50-53 and 57-60. The synthesis of compounds **50-53** and **57-60** is illustrated with specific reference to compounds **50** and **57**, the other compounds being analogously synthesizable. A mixture of the acid **45** (1.5 g, 1.0 equiv.) and HBTU (1.8 g, 1 equiv.) in DMF (8 mL) and DIEA (2 mL) was stirred at RT for 1hr, treated with the 4-
15 (2-aminoethyl)morpholine (0.70 mL, 1.1 equiv.) and stirred for 15 hr at RT. The solution was added dropwise to ice water (150 mL) and extracted with AcOEt (5x). The combined organic layers were dried (MgSO₄) and evaporated to give compound **50** as a brown solid (1.6 g, ¹H-NMR). The crude product was dissolved in AcOEt (50 mL) and MeOH (5 mL), treated with 10% Pd-C (*ca.* 100 mg), and stirred at RT under H₂ (1 atm) for 48 hr.
20 The mixture was filtered through *Celite* and the solids washed with MeOH. The filtrate was concentrated *in vacuo*, diluted with Et₂O (250 mL) and AcOEt (50 mL), and treated with HCl (g) for 1 min. Evaporation of the solvents gave compound **57** hydrochloride as an orange solid (1.7 g), used without further purification.

Example D

25 [99] In this example, intermediates synthesized per Examples A and C are coupled to provide compounds of this invention. The synthetic scheme is summarized in Scheme D-1a, with compound **Ib-56** as a representative example.

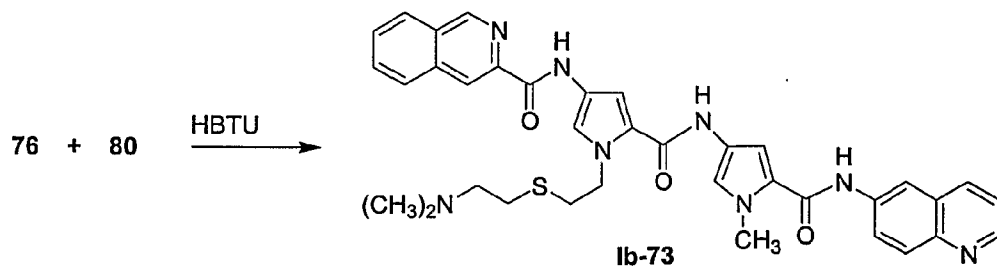
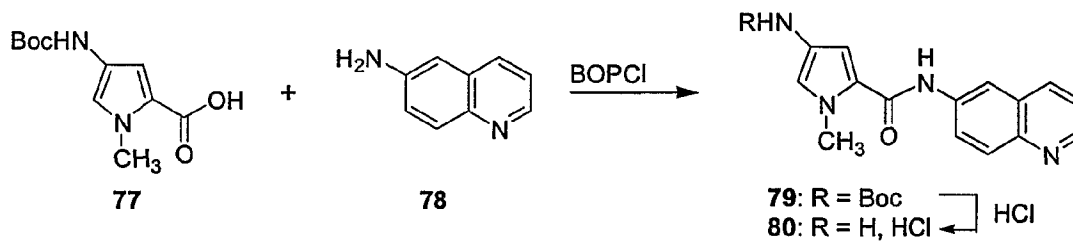
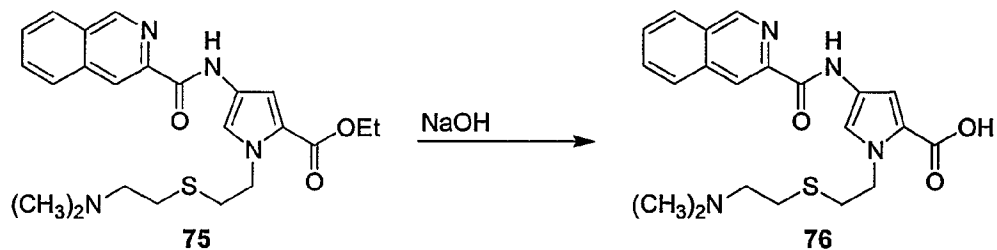
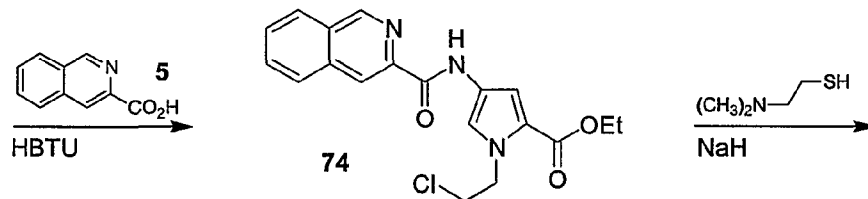
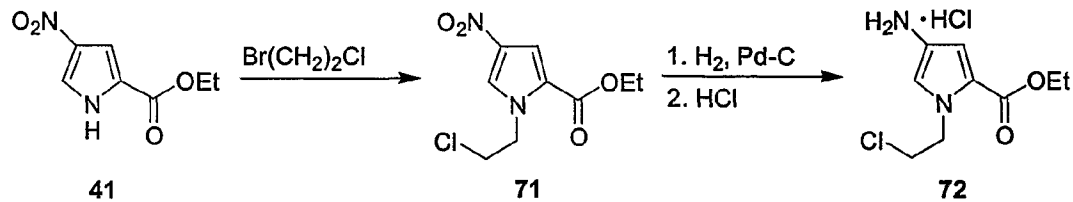
Scheme D-1

[100] **Compound Ib-56.** Acid 19 (108.5 mg, 1.2 equiv.) and amine-amide 57 (100 mg, 1.0 equiv.) were coupled using HBTU/DIEA/NMP to give, after purification by HPLC, compound Ib-56 (¹H-NMR, mass spectrum). Compounds Ib-55, Ib-64, and Ib-68 were made by analogous methods.

Example E

[101] Scheme E-1 illustrates the synthesis of compounds having basic pendant side chains such as those found in compound Ib-73.

Scheme E-1



- 10 [102] Nitro ester 71. A mixture of the pyrrole 41 (Example C) (20.00 g, 1.0 equiv.), NaI (16.28 g, 1.0 equiv.), K_2CO_3 (30.02 g, 2.0 equiv.) and 1-bromo-2-chloroethane (22.6 mL, 2.5 equiv.) in DMF (200 mL) was stirred at 75°C for 2.5 hr and poured into H_2O (500

mL). The mixture was treated with Na₂S₂O₃ (ca. 10g) and extracted with Et₂O (3x, each 300 mL). The organic layers were dried (MgSO₄) and evaporated to give a yellow oil. Drying at 75°C (ca. 0.1 mbar) for 1.5 hr gave nitro ester **71** (26.77 g, 93%, ¹H-NMR) as a crystalline yellow solid.

- 5 [103] Amino ester 72. A suspension of the nitro ester **71** (15.0 g, 1 equiv.) and 10% Pd/C (1.0 g) in AcOEt (117 mL) and MeOH (13 mL) was stirred at RT under H₂ (120 psi) for 20 hr. The mixture was filtered through Celite and the solvent was evaporated from the filtrate. The resulting brown oil was dissolved in Et₂O (ca. 300 mL) and treated at 0°C with HCl (g) for about 45 sec. The resulting precipitate was collected by filtration and
10 dried to give amino ester **72** hydrochloride as a tan solid (14.03 g, 91%, ¹H-NMR).

[104] Isoquinoline ester 74. Isoquinoline-3-carboxylic acid **5** (Example A) (3.76 g, 1.1 equiv.) and amino pyrrole **72** (5.00 g, 1.0 equiv.) were coupled using HBTU/DIEA/DMF to give isoquinoline ester **74** as a tan solid (5.90 g, 80%, ¹H-NMR), isolated by filtration.

- [105] Compound 75. A suspension of 2-(dimethylaminoethyl)mercaptan hydrochloride
15 (350 mg, 2 equiv.) in DMF (5 mL) was treated at 0°C under N₂ with NaH (60% disp., 200 mg, 4 equiv.). The mixture was stirred for 20 min at 0°C, treated dropwise with a solution of isoquinoline ester **74** (0.458 g, 1 equiv.) in DMF (5 mL), warmed to RT and stirred for 1 hr. The mixture was diluted with AcOEt (200 mL) and washed with sat. aqueous K₂CO₃ (2x, each 30 mL) and H₂O (1x, 30 mL). The organic layer was dried (MgSO₄) and
20 evaporated to give compound **75** as a dark solid (0.45 g, 83%, ¹H-NMR).

[106] Acid 76. Saponification of compound **75** (0.45 g, 1 equiv.) with NaOH/aq. MeOH at 60 °C for 3 hr gave acid **76** (0.40 g, 95%, ¹H-NMR).

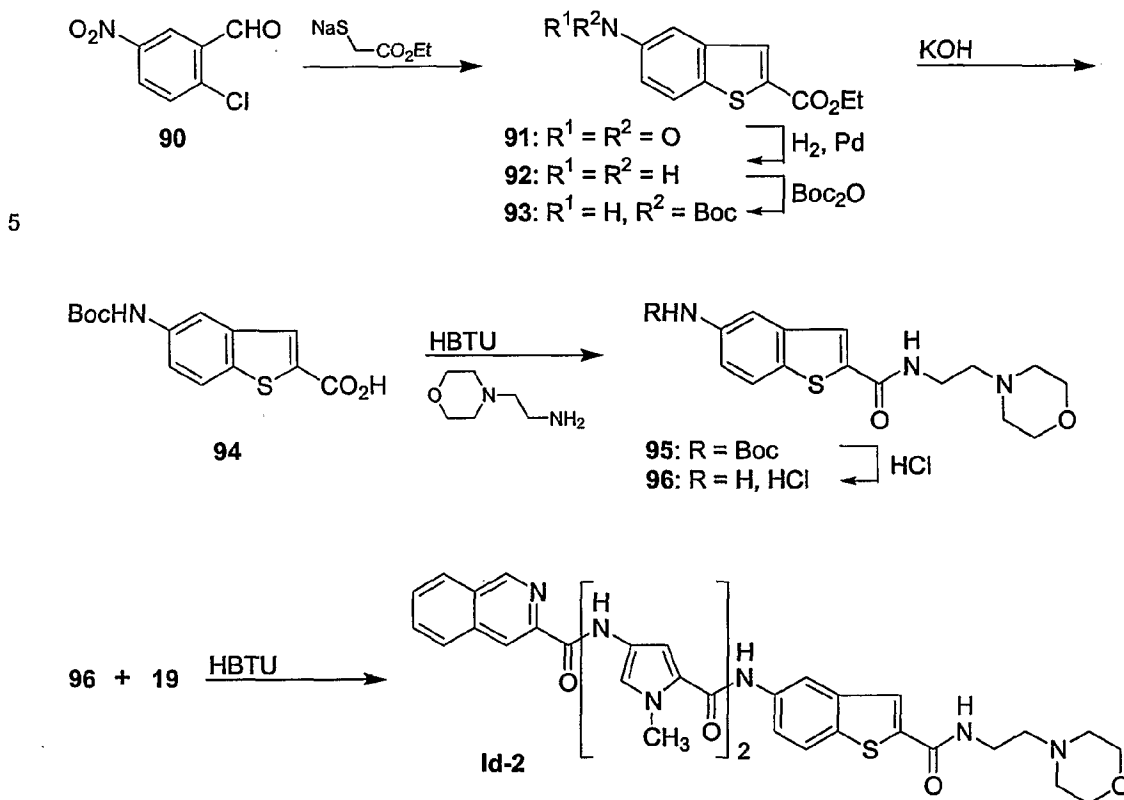
- [107] Compound 79. Acid **77** (5.00 g, 1.0 equiv.) and 6-aminoquinoline **78** (3.00 g, 1.0 equiv.) were coupled using BOPCl/DIEA/NMP to give compound **79** (3.73 g, ca. 49%),
25 used without further purification or characterization.

[108] Amine 80. A suspension of compound **79** (3.41 g, 1.0 equiv.) in AcOEt (100 mL, saturated with HCl gas) was stirred at 0°C for 3 hr and poured into Et₂O (300 mL). The solid was collected by filtration and dried in vacuo to give amine **80** as a dark solid (3.09 g). The crude product was used without further purification or characterization.

- 30 [109] Compound Ib-73. Carboxylic acid **76** (50 mg, 1.1 equiv.) and amine **80** were coupled using HBTU/DIEA/NMP to yield compound **Ib-73** (¹H-NMR, mass spectrum).

Example F

[110] This example illustrates the synthesis of compounds having condensed carboxamide moieties having condensed 6,5-heteroaromatic ring systems, as illustrated by compound **Id-2**. The synthetic approach is summarized in Scheme F-1.

Scheme F-1

10 [111] Nitro ester 91. A mixture of the nitrobenzaldehyde **90** (3.00 g, 1.0 equiv.), sodium ethyl mercaptoacetate (3.10 g, 1.35 equiv.) and Na₂CO₃ (1.80 g, 105 equiv.) in DMSO (30 mL) was stirred at 90 °C for 15 hr and poured into H₂O (350 mL). The solution was extracted with Et₂O (4x). The combined organic layers were dried (MgSO₄) and evaporated to give the nitro ester **91** as a yellow-brown crystalline solid (3.60 g, 89%,
 15 ¹H-NMR).

[112] Amino ester 92. A suspension of the nitro ester **91** (2.50 g, 1.0 equiv.) and 10% Pd/C (200 mg) in AcOEt (75 mL) and MeOH (8 mL) was stirred at RT under H₂ atmosphere (150 psi) for 19 hr. The mixture was filtered through *Celite*. Evaporation of

the solvent gave amino ester **92** as a yellow solid, used without further purification or characterization.

[113] Boc-protected ester 93. Crude amino ester **92** was dissolved in DMF (16 mL) and DIEA (4 mL), treated with (Boc)₂O (2.08 g, 1.5 equiv.) and stirred at RT for 8 hr. The mixture was poured into H₂O (300 mL) and extracted with Et₂O (4x). The combined organic layers were dried (MgSO₄) and evaporated to give Boc-protected ester **93** as a pale yellow solid (2.70 g, 81% yield over two steps, ¹H-NMR).

[114] Acid 94. Saponification of the ester **93** (2.70 g, 1 equiv.) using KOH/aq. EtOH at 65 °C for 4 hr gave acid **94** as a tan solid (2.28 g, 95%, ¹H-NMR).

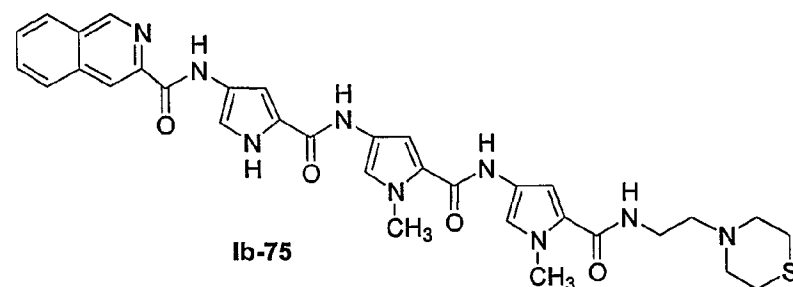
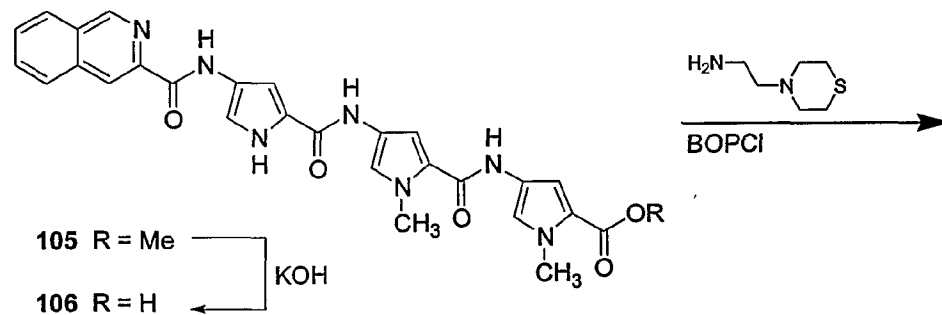
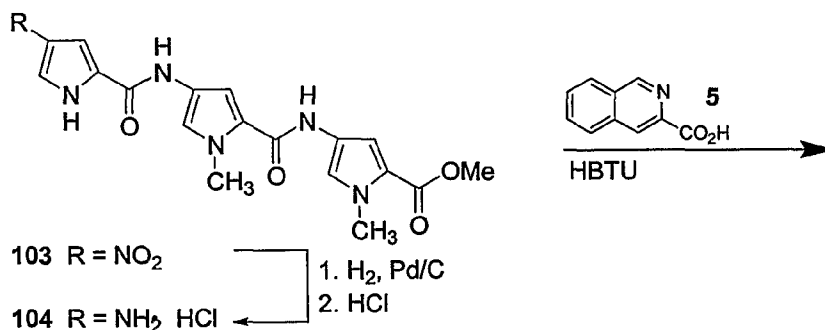
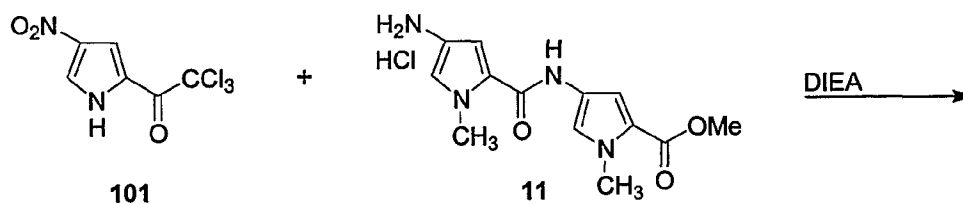
[115] Amine 96. Acid **94** (600 mg, 1.0 equiv.) and 4-(2-aminoethyl)morpholine (280 mg, 1.05 equiv.) were coupled using HBTU/DIEA/NMP to give compound **95** as an orange tar. Crude compound **95** was dissolved in AcOEt (50 mL) and saturated with HCl (g) and stirred for 2 hr at RT. The resulting precipitate was collected by filtration and dried to give amine **96** as a tan solid (ca. 550 mg).

[116] Compound Id-2. Acid **19** (Example A) (79.6 mg, 1.2 equiv.) and amine **96** (60 mg, 1.0 equiv.) were coupled using HBTU/DIEA/NMP to give, following HPLC purification, compound **Id-2** (¹H-NMR, EI-mass spectrum).

Example G

[117] This example illustrates the synthesis of compounds having an N-unsubstituted pyrrole carboxamide unit, examples of which are **Ib-50**, **Ib-54**, **Ib-62**, **Ib-67** and **Ib-69** to **Ib-71**.

[118] Scheme G-1 illustrates, with compound **Ib-75** as a model, the procedure for the instance in which an N-unsubstituted pyrrole carboxamide group has two consecutive Py groups attached to its carboxyl side.

Scheme G-1

[119] Trimer 103. A mixture of ketone **101** (Bremer et al., Bioorg. Med. Chem. **2000**, 8, 1947-1955) (6.00 g, 1 equiv.) and amine **11** (Example A) (7.27 g, 1 equiv.) in NMP (50 mL) and DIEA (9.5 mL) was stirred for 2 hr at RT and added dropwise to ice water (800 mL). The resulting solid was collected by filtration and dried *in vacuo* to give the trimer **103** (9.40 g, 97%, ¹H-NMR).

[120] Amine 104. Hydrogenation of trimer **103** (1.19 g, 1 equiv.) at 100 psi H₂ with 10% Pd-C (0.2 g) catalyst in AcOEt:MeOH (1:1 v/v) for 16 hr gave amine **104** hydrochloride (1.0 g, 83%, ¹H-NMR), collected by filtration after precipitation with HCl.

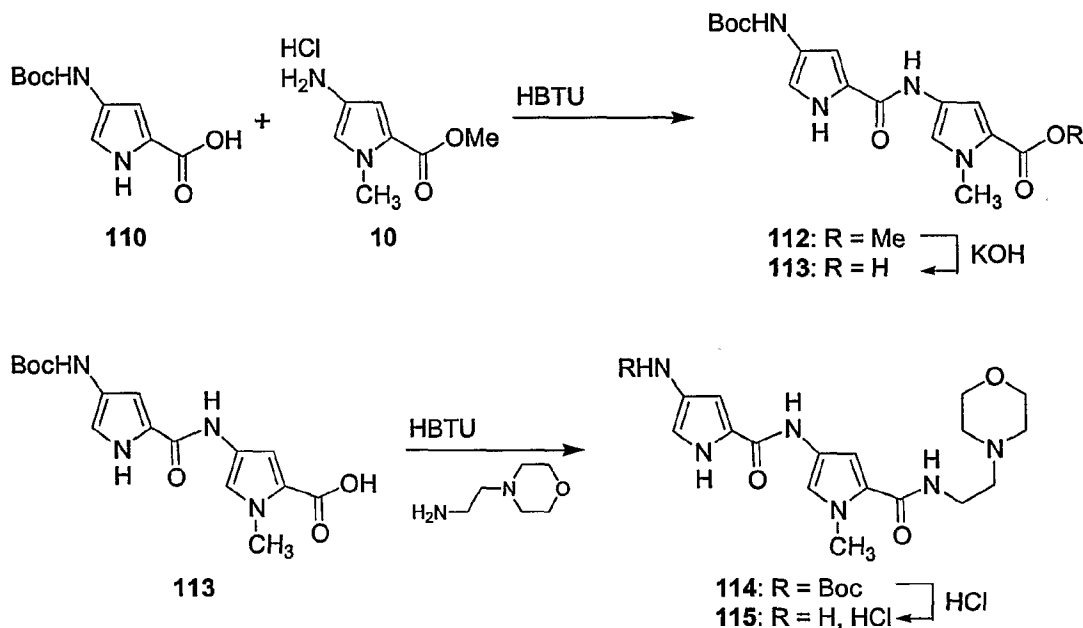
[121] Tetrameric ester 105. Isoquinoline-3-carboxylic acid **5** (1.33 g, 1.2 equiv.) and amine **104** (2.90 g, 1.0 equiv.) were coupled using HBTU/DIEA/NMP to give, after work-up and collection by filtration, tetrameric ester **105** (3.5 g, 96%, ¹H-NMR).

[122] Tetrameric acid 106. Saponification of tetrameric ester **105** (3.50 g, 1 equiv.) with KOH/aq. EtOH for 8 hr at 60°C gave, after acidification and work-up, tetrameric acid **106** (2.34 g, 68%, ¹H-NMR).

[123] Compound Ib-75. Tetrameric acid **106** (80 mg, 1 equiv.) and 4-(2-aminoethyl)-thiomorpholine (0.1 mL) were coupled using HBTU/DIEA/NMP (65 mg, 1.1 equiv.) to give, after preparative HPLC, compound **Ib-75** (¹H-NMR, mass spectrum).

[124] Scheme G-2 below illustrates the preparation intermediates for the synthesis of compounds such as **Ib-67**, wherein an N-unsubstituted pyrrole carboxamide has a single Py group attached to its carboxyl side.

Scheme G-2



[125] Dimer 112. Acid **110** (19.58 g, 1.1 equiv.) and amine **10** (Example A) (15.0 g, 1.0 equiv.) were coupled using HBTU/DIEA/DMF to give dimer **112** as an orange brown oil (¹H-NMR).

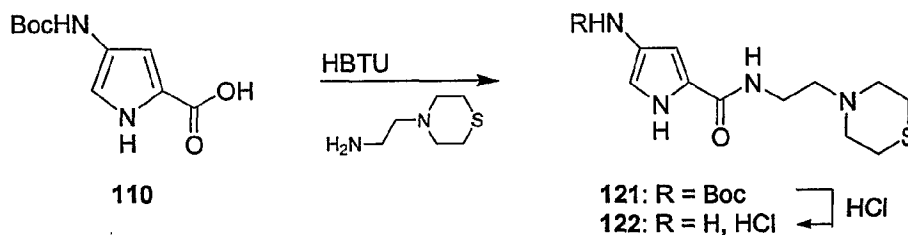
[126] Acid 113. Saponification of the dimer 112 (10.0 g, 1 equiv.) in KOH/EtOH/H₂O gave, after acidification and work-up, acid 113 as a brown solid (¹H-NMR).

[127] Amine 115. Acid 113 (4.00 g, 1.1 equiv.) and 4-(2-aminoethyl)morpholine (1.37 mL, 1.0 equiv.) were coupled using HBTU/DIEA/DMF to give intermediate 114 (mass spectrum). Crude intermediate 114 was dissolved in AcOEt:MeOH (2:1 v/v), saturated with HCl (g) and stirred for 1.5 hr. Evaporation of the solvent left a brown oil that was dissolved in MeOH (25 mL) with sonication. The solution was added dropwise to Et₂O (250 mL) upon which a brown solid precipitated. Decantation of the solvent and drying gave amine 115 (¹H-NMR).

10 Example H

[128] Scheme H-1 below describes the synthesis of a building block for compounds having an N-unsustituted pyrrole carboxamide group at the C-terminus, such as compound Ib-54.

Scheme H-1

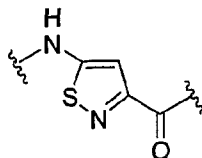


15 [129] Amine 122. Acid 110 (Scheme G-2) (2.00 g, 1.0 equiv.) and 4-(2-aminoethyl)thiomorpholine (1.68 g, 1.3 equiv.) were coupled using HBTU/DIEA/DMF to give, after flash chromatography (1%TEA, CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) gradient), intermediate 121 as a glassy yellow solid (320 mg). Intermediate 121 was dissolved in AcOEt (30 mL), treated with HCl-saturated AcOEt (75 mL) and stirred for 2 hr at RT.

20 Evaporation of the solvent left amine 122 hydrochloride as a white solid (300 mg, ¹H-NMR, mass spectrum), used to make compound Ib-54.

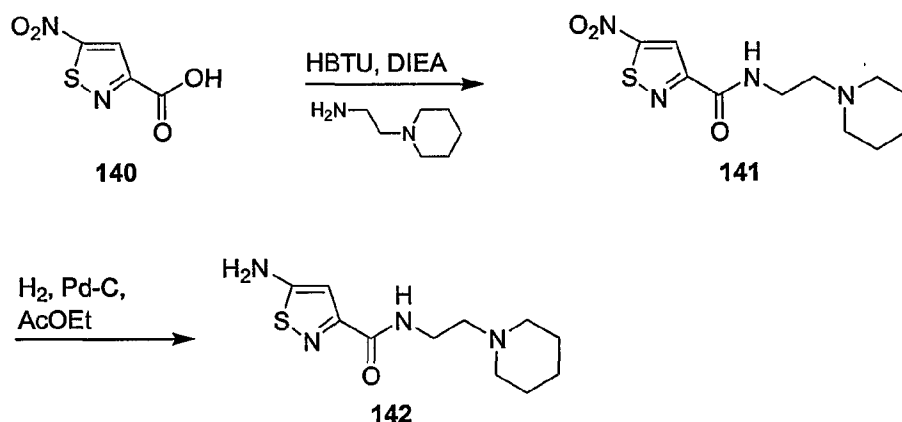
Example I

[130] Scheme I-1 below illustrates the synthesis of intermediates usable for the preparation of compounds of this invention having isothiazole carboxamide residues



as exemplified by compounds Ib-52 and Ib-53.

Scheme I-1

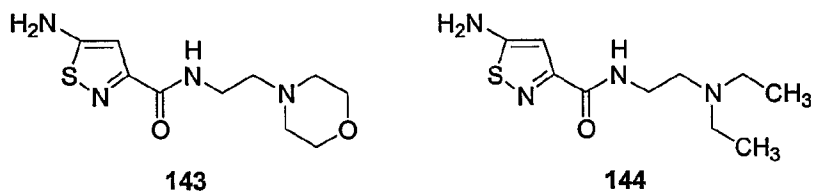


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[131] Nitro compound 141. Nitro acid 140 (300 mg, 1.2 equiv., Heindl et al., *Eur. J. Med. Chem. – Chimica Therapeutica*, 1975, 10, 591-593) and 1-(2-aminoethyl)piperidine (204 μ L, 1.0 equiv.) were coupled using HBTU/DIEA/DMF to give nitro compound 141 (400 mg, 98%, ¹H-NMR).

10 [132] Amine 142. Nitro compound 141 (400 mg) was hydrogenated under 1 atm H₂ using 10% Pd-C (ca. 500 mg) in AcOEt (30 mL) at RT for 24 hr to give amine 142 as a light yellow powder (110 mg, 27%, ca. 90 to 95% pure by ¹H-NMR). Without further purification, this material was used for the synthesis of compounds Ib-52 and Ib-53.

[133] Analogous compounds such as 143 and 144

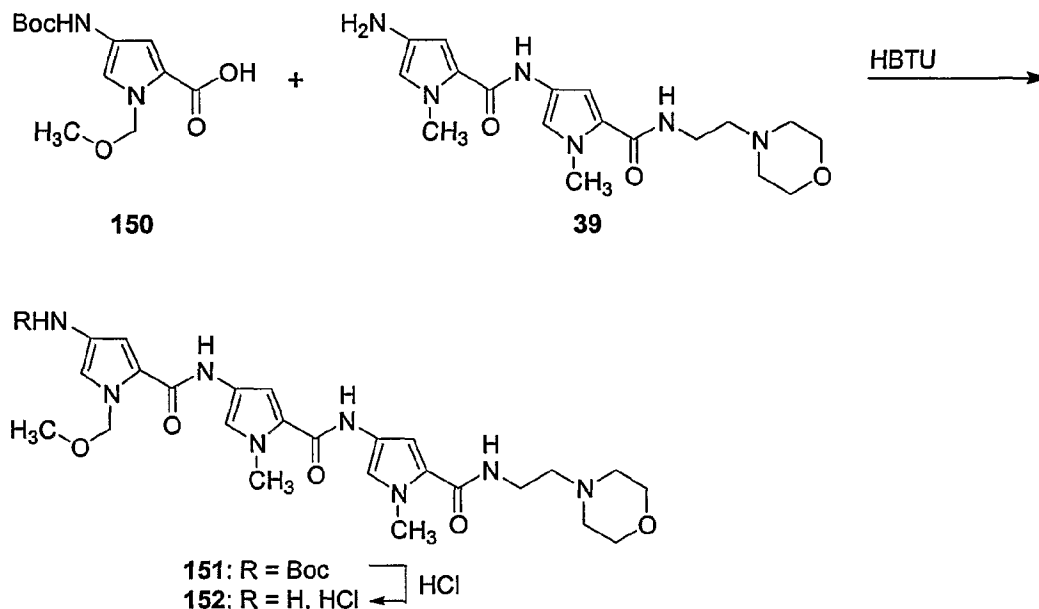


15

were prepared by replacing the 1-(2-aminoethyl)piperidine with a different amine.

Example J

[134] Scheme J-1 below describes the preparation of an intermediate for the synthesis of compounds such as **Ib-72**.

Scheme J-1

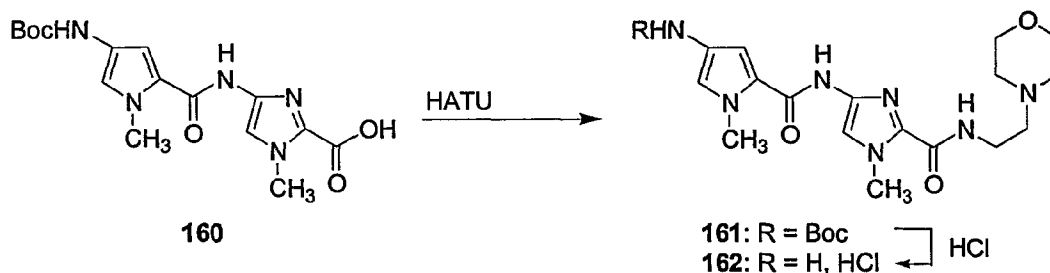
[135] Acid 150. Acid **150** was prepared by alkylation of nitro pyrrole ester **41** (Example C) with MOMCl followed by hydrogenation (H_2 , Pd) and Boc-protection (Boc_2O). The Boc-protected amino ester was saponified to give acid **150**.

10 [136] Compound 152. Acid **150** (1.66 g, 1.1 equiv.) and amine **39** (Example B) (2.50 g, 1.0 equiv.) were coupled using HBTU/DIEA/DMF to give, after flash chromatography (CH_2Cl_2 and 0 to 15% MeOH gradient) of the crude product, compound **151** (1.7 g) as a glassy solid. Compound **151** was dissolved in AcOEt (50 mL), treated with HCl-saturated AcOEt (50 mL) and stirred at 0 °C for 1.5 hr. Evaporation of the solvent gave compound

15 **152** as a tan solid (1.49 g, mass spectrum). The 1H -NMR was consistent with assigned structure, but showed signals of minor impurities, which were not further characterized.

Example K

[137] Scheme K-1 below depicts the synthesis of amine **162**, used in the synthesis of compound **Ib-58**.

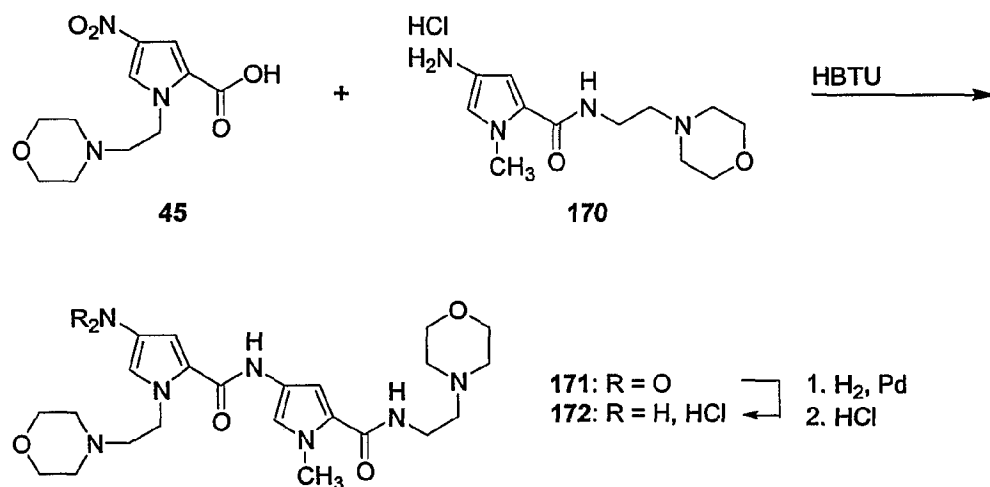
Scheme K-1

[138] Compound 161. A mixture of dimeric acid **160** (US 6,090,047) (0.50 g, 1.2 equiv.) and HATU (0.496 g, 1.14 equiv.) in DMF (5 mL) and DIEA (0.5 mL) was stirred at RT for 30 min and treated with 4-(2-aminoethyl)morpholine (150 mg, 1 equiv.). The mixture was stirred for 21 hr at RT, diluted with H₂O (ca. 150 mL) and extracted with AcOEt (4 x 50 mL). The combined organic layers were dried (MgSO₄) and evaporated to give compound **161** (ca. 640 mg, ¹H-NMR (minor impurities)), used without further purification.

[139] Amine 162. A solution of compound **161** (640 mg, 1.0 equiv.) in AcOEt (20 mL) and Et₂O (15 mL) was saturated at RT with HCl (g). The resulting solids were collected by filtration and dried to give amine **162** (¹H-NMR).

Example L

[140] Scheme L-1 below shows the synthesis of intermediate amine **172**, used in the preparation of compound **Ib-57**.

Scheme L-1

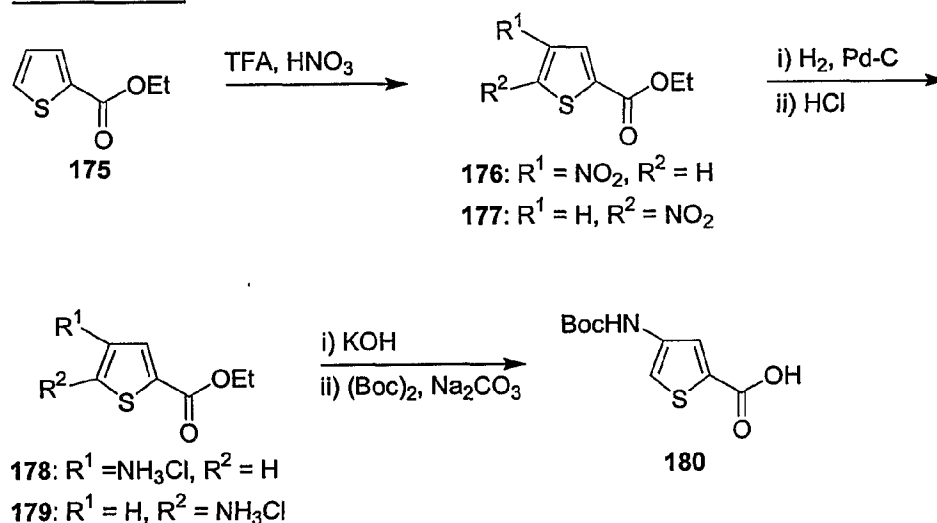
[141] Amine 172. Nitro acid **45** (Example C) (1.05 g, 1.1 equiv.) and amine **170** (prepared from acid **77** (Example E) by analogy to the conversion of compound **30** to compound **39** in Example B) (1.00 g, 1.0 equiv.) were coupled using HBTU/DIEA/DMF to give compound **171** as a brown tar. Hydrogenation of crude compound **171** (150 psi H₂, 10%Pd-C, AcOEt:MeOH (9:1 v/v), RT, 23 hr) gave amine **172** as a brown solid (¹H-NMR), isolated as the hydrochloride salt.

[142] Amine **172** was reacted with dimeric acid **18** (Example A) using HBTU/DIEA in NMP to yield compound **Ib-57** (¹H-NMR, mass spectrum).

Example M

[143] Scheme M-1 below summarizes the synthesis of a carboxamide building block having a thiophene ring, suitable for making compounds such as **Ib-51**.

Scheme M-1



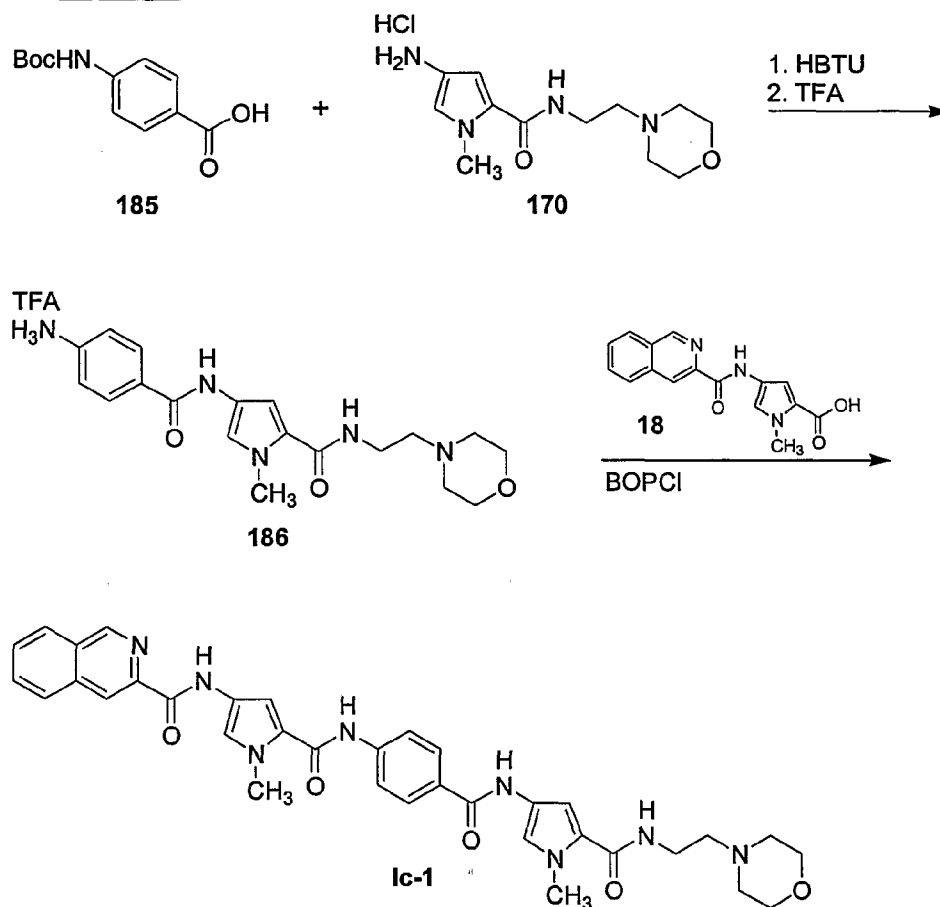
[144] Nitro esters 176/177. A solution of ethyl-2-thiophene carboxylate **175** (200 g, 1 mol) in TFA (200 mL) was slowly added to a mixture of TFA (900 mL) and fuming nitric acid (200 mL) at 5°C. The cooling was removed and the reaction mixture stirred at 45°C for ca. 14 hr, cooled to ca. 10°C, and poured into vigorously stirred ice water (4 L). The resulting precipitate was collected by filtration and washed with ice water (2x). Lyophilization of the resulting solids gave a mixture of nitro esters **176** and **177** (2:3, as evidenced by ¹H-NMR, 194.2 g, 79%).

[145] Amines 178/179. A mixture of nitro esters **176** and **177** (2:3, 20g) was hydrogenated (100 psi H₂, 10% Pd-C, EtOAc:MeOH (9:1 v/v), RT, 6 days) to give a mixture of amines **178** and **179** (0.9:1 as evidenced by ¹H-NMR, 19.02 g, 93%).

[146] Acid 180. Amine **178** was selectively saponified by treating a mixture of amines **178** and **179** (0.9/1, 15 g) in methanol (500 mL) at 0°C with a solution of KOH (9 g) in H₂O (75 mL), with stirring, for 3 hr. The reaction mixture was then diluted with H₂O (400 mL) and washed with EtOAc (3x, 200 mL). The aqueous layer was neutralized to pH 6.5 with a 1M aq. HCl solution, treated with Na₂CO₃ (15 g) and a solution of Boc-anhydride (15 g) in dioxane (150 mL) and stirred for 24 hr at RT. The reaction mixture was washed with EtOAc (3x, 200 mL), cooled to 0°C, acidified to pH 2.8 with aqueous HCl (50%), and extracted with EtOAc (3x, 200 mL). The combined organic layers were dried (MgSO₄) and evaporated. The remaining oil was dissolved in methanol and treated with activated carbon (5 g). The mixture was filtered through Celite and the filtrate evaporated to yield acid **180** free of the other isomers.

15 Example N

[147] This example describes the synthesis of compound **Ic-1**, representative of molecules containing an amino benzamide unit. The synthetic route is summarized in Scheme N-1. Compounds having other C-terminal amino groups, such as **Ic-4**, can be prepared analogously.

Scheme N-1

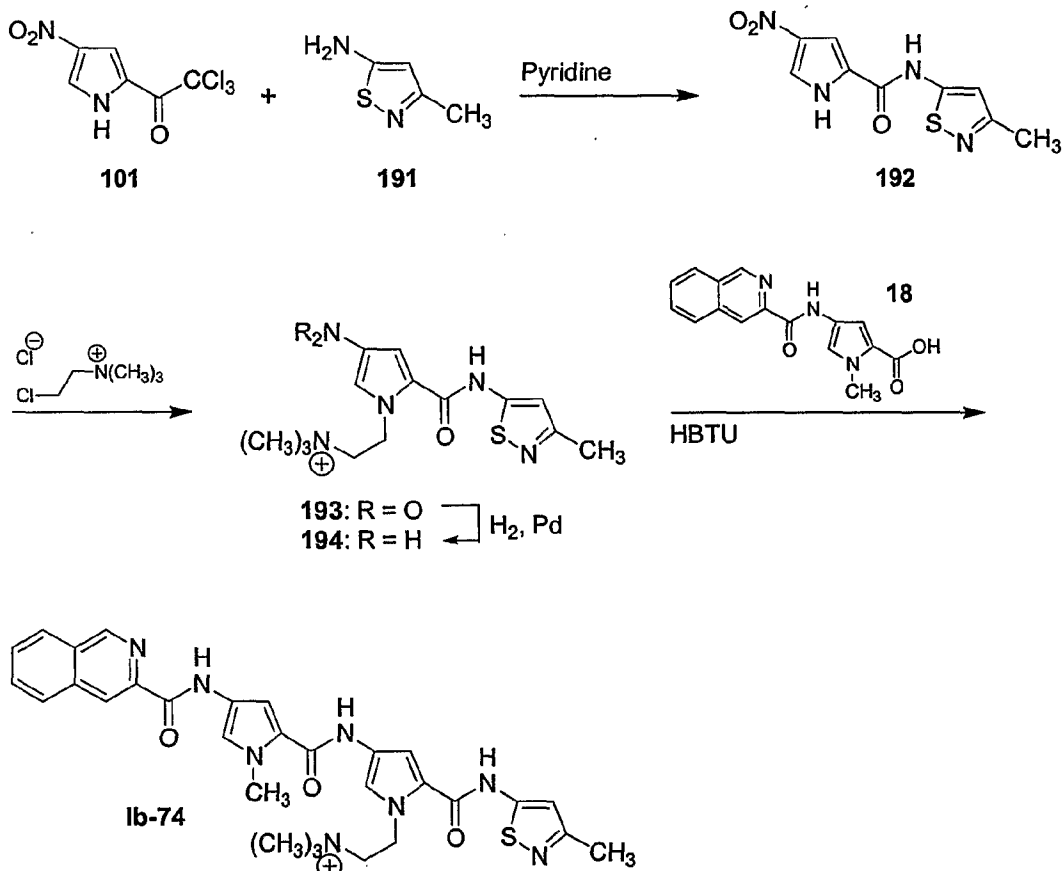
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[148] Amine 186. 4-Boc-aminobenzoic acid **185** (0.85 g, 3.6 mmol) and amine **170** (Example L) were coupled using HBTU/DIEA/NMP to give crude Boc-protected product. Treatment with TFA (10 mL, excess) at room temperature for 1 hour removed the Boc group. Volatile components were then removed under vacuum to give amine **186** as the TFA salt, which was used without further purification.

[149] Compound Ic-1. BOPCl (140 mg, 0.55 mmol) and acid **18** (Example A) (177 mg, 0.6 mmol) were dissolved in NMP (4 mL) and DIEA (0.7 mL, 2 mmol) and the reaction stirred at 40°C for 30 minutes. Amine **186** (0.5 mL, 1M solution in NMP, 0.5 mmol) was then added and the reaction shaken at 60°C overnight. The reaction was diluted in 40% acetic acid solution to a total volume of 15 mL and the product obtained from HPLC purification of the reaction mixture. Compound **Ic-1** was obtained as a pale yellow solid (¹H-NMR).

Example O

[150] The synthesis of a compound of this invention having a quaternized nitrogen group is illustrated, with specific reference to compound **Ib-74** (Scheme O-1).

Scheme O-1

[151] Compound 192. A mixture of the trichloromethyl ketone 101 (Example G) (50.00 g, 1.05 equiv.) and 5-amino-3-methylisothiazole 191 (29.15 g, 1.0 equiv.) in DMF (300 mL) and pyridine (75 mL) was stirred at 65°C for 22 hr. The mixture was poured into ice-water (3.6 L) and the resulting precipitate collected by filtration, washed with H₂O and dried *in vacuo* to give compound 192 as a light tan solid (48.9 g, > 95%, ¹H-NMR).

[152] Compound 193. A mixture of the compound 192 (2.00 g, 1.0 equiv.), 2-chloroethyltrimethylammonium chloride (3.14 g, 2.5 equiv.), NaI (1.19 g, 1.0 equiv.) and K₂CO₃ (3.29 g, 3.0 equiv.) in NMP (80 mL) was stirred at 65-80°C for 24 hr, treated with additional chloroethyltrimethylammonium chloride (1.2 g) and stirred for 3 hr at 80°C. The mixture was diluted with H₂O (150 mL) and washed with AcOEt (6x). The

crystalline material formed in the aqueous phase was collected by filtration to give compound **193** as orange crystals (360 mg, 13%, ¹H-NMR, mass spectrum).

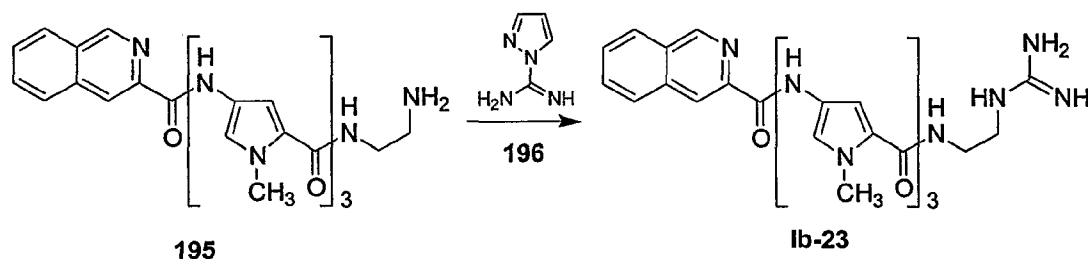
[153] Compound 194. Compound **193** (360 mg, 1 equiv.) was hydrogenated (1 atm H₂, 10%Pd-C, AcOEt:MeOH (1:9 v/v), RT, 7 hr) to give compound **194** as an orange solid (1H-NMR, mass spectrum).

[154] Compound Ib-74. Carboxylic acid **18** (Example A) (50.3 mg, 1.3 equiv.) and compound **194** (45 mg, 1.0 equiv.) were coupled using HBTU/DIEA/NMP to give, after purification by HPLC, compound **Ib-74** (¹H-NMR, mass spectrum), presumably as the acetate salt.

10 Example P

[155] This example describes a generally applicable protocol for the guanidylation of primary amines, using compound **Ib-23** as a model.

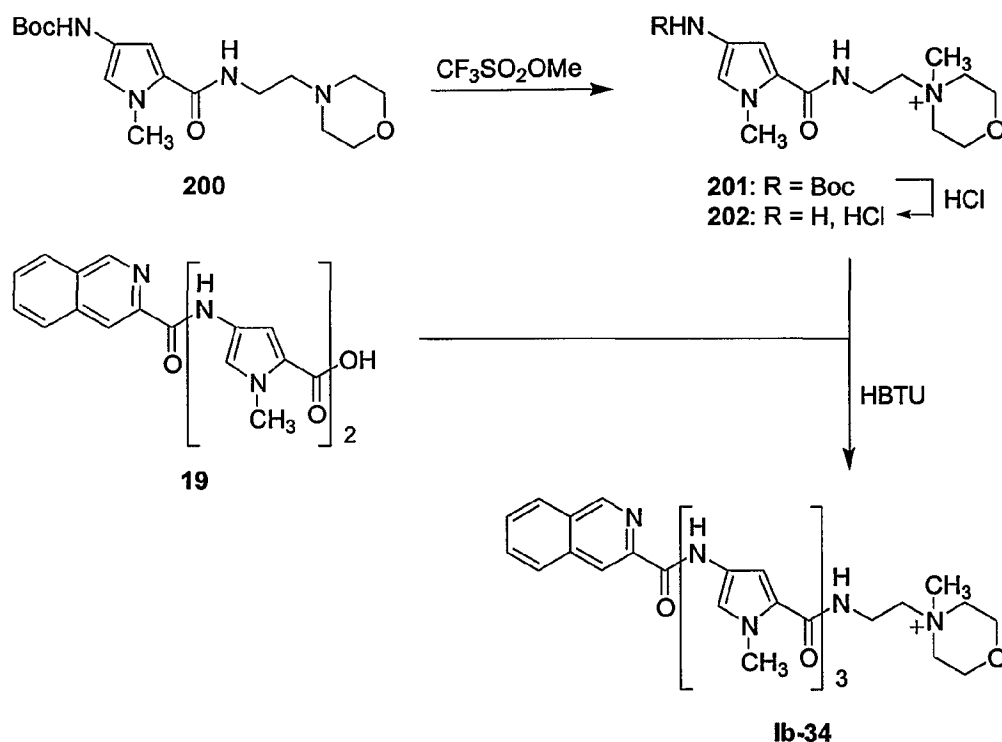
Scheme P-1



[156] A mixture of primary amine **195** and pyrazole **196** (ca. 6 equiv.) in DMF and DIEA was stirred at RT for ca. 16 hr. The reaction mixture was diluted with 50% aqueous AcOH and purified by HPLC to yield compound **Ib-23** (¹H-NMR, mass spectrum).

Example Q

[157] This example illustrates the synthesis of a compound having quaternized nitrogen group at the C-terminus, with specific reference in Scheme Q-1 to compound **Ib-34**.

Scheme Q-1

[158] **Compound 201.** A solution of compound **200** (1.00 g, 1.0 equiv.) in CH_2Cl_2 (10 mL) was treated at RT with methyl triflate (0.337 mL, 1.05 equiv.) and stirred for 2 hr. The mixture was treated with DIEA (0.5 mL) and additional methyl

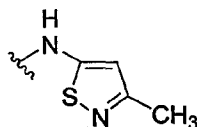
trifluoromethanesulfonate (0.3 mL). Stirring was continued for 1.5 hr and the solvent was evaporated. The ^1H -NMR of the crude product was in agreement with the structure of compound **201**, but showed minor impurities. The material was used without further purification.

[159] **Compound 202.** A solution of crude compound **201** in MeOH (ca. 40 mL) was saturated with HCl (g) for about 20 seconds and stirred at RT for 1 hr. Evaporation of the solvent gave compound **202** (^1H -NMR, mass spectrum).

[160] **Compound Ib-34.** Carboxylic acid **19** (Example A) (88.7 mg, 1.2 equiv.) and compound **202** (60 mg, 1.0 equiv.) were coupled using HBTU/DIEA/NMP to give, after purification by HPLC, compound **Ib-34** (^1H -NMR, mass spectrum), presumably as the acetate salt.

Example R

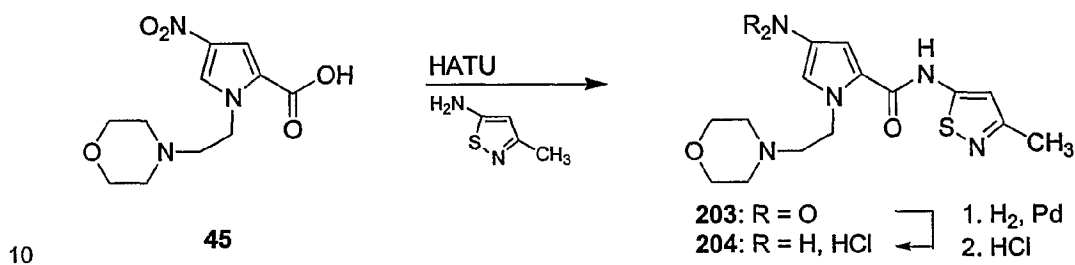
[161] This example relates to compounds in which $-\text{Z}(\text{R}^2)_n$ is



(e.g., **Ib-65**, **Ib-66** and **Ib-74**). Generally, they are made by coupling commercially available 5-amino-3-methylisothiazole with a complementary carboxylic acid intermediate. The coupling preferably is effected with HATU, which produces a more reactive activated ester than HBTU, compensating for the diminished reactivity of the isothiazole amine group. Also, it may be desirable to run the coupling reaction at a higher temperature.

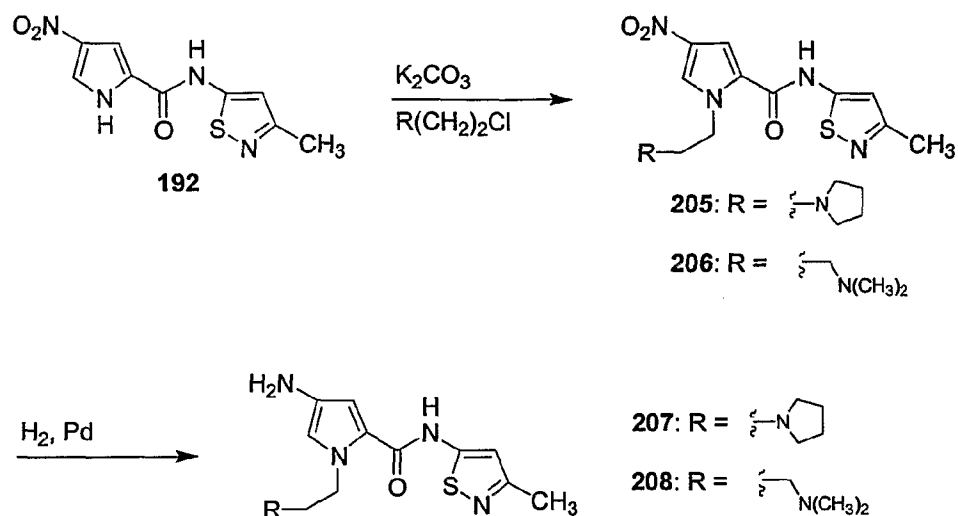
[162] Scheme R-1 below shows a procedural variant employed in the synthesis of compound **Ib-63**.

Scheme R-1



[163] **Compound 204.** A mixture of the carboxylic acid **45** (Example C) (1.57 g, 1.0 equiv.) and HATU (2.21 g, 1.0 equiv.) in DMF (8 mL) and DIEA (1.5 mL) was stirred at RT for 30 min and added to a solution of 5-amino-3-methylisothiazole (0.88 g, 1.0 equiv.) in DMF (5 mL) and DIEA (1 mL). The mixture was stirred for 8 hr at 60°C and 12 hr at RT, treated with H₂O and extracted with AcOEt (3x). The combined organic layers were dried (MgSO₄) and evaporated to give compound **203** (¹H-NMR). Crude compound **203** was hydrogenated (100 psi H₂, 10% Pd-C, AcOEt:MeOH (1:1 v/v), RT, 24 hr) to give compound **204**, used without further purification.

[164] Scheme R-2 below shows another procedural variation, applicable for making
20 precursors of compounds such as **Ib-65** and **Ib-66**.

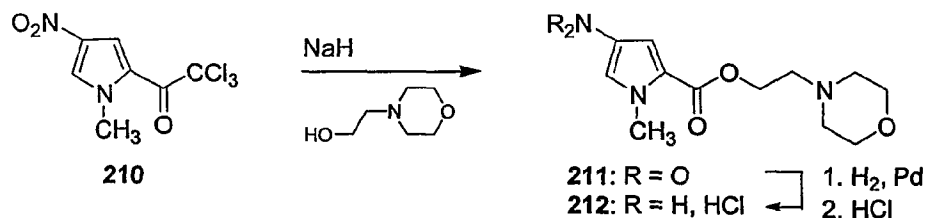
Scheme R-2

[165] Compounds 205 and 207. A mixture of the dimer **192** (Example O) (3.0 g, 1.0 equiv.), 1-(2-chloroethyl)pyrrolidine hydrochloride (2.83 g, 1.4 equiv.), NaI (1.96 g, 1.1 equiv.) and K_2CO_3 (3.29 g, 2.0 equiv.) in DMF (30 mL) was stirred at 65°C for 23 hr, treated with additional 1-(2-chloroethyl)pyrrolidine hydrochloride (3.0 g) and stirred for 24 hr. The reaction mixture was poured into a mixture of H_2O (150 mL) and sat. aqueous K_2CO_3 and extracted with AcOEt (5x). The combined organic layers were dried (MgSO_4) and evaporated to give a tar (3.36 g). The $^1\text{H-NMR}$ indicated the presence of two products. The material was used without further purification. Crude compound **205** was hydrogenated (150 psi H_2 , 10%Pd-C, AcOEt/MeOH (1:1 v/v), RT, 19 hr) to give compound **207** as a tan solid (3.09 g).

[166] Compounds **206** and **208** were analogously synthesized.

Example S

[167] Scheme S-1 below relates to the synthesis of intermediates for the preparation of compounds of this invention having a C-terminal ester group.

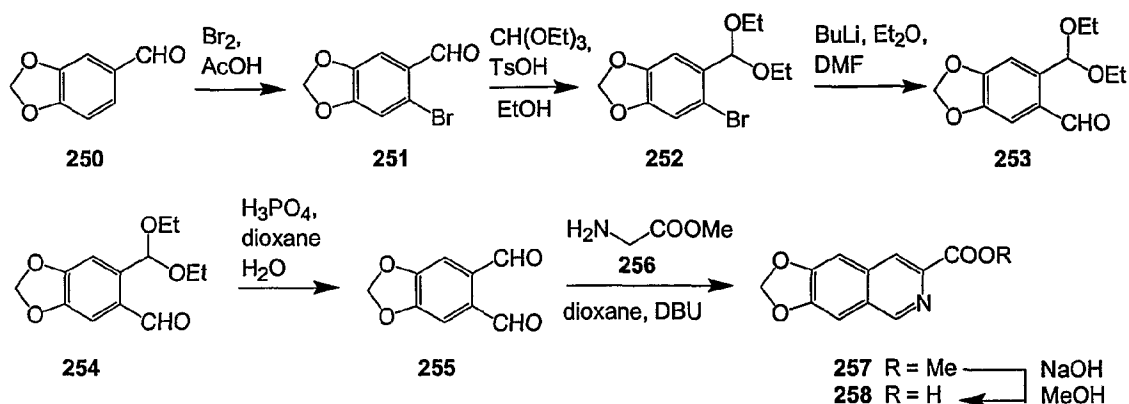
Scheme S-1

[168] Amine 212. A solution of 4-(2-hydroxyethyl)morpholine (3.4 mL, 3.0 equiv.) in THF (20 mL) was treated at 0°C portionwise with NaH (0.37 g, 1.0 equiv., 60% in dispersion). The mixture was stirred for 20 min until a clear solution was observed and treated with a solution of the trichloromethyl ketone **210** (Baird et al., J. Am. Chem. Soc. 1996, 118, 6141-6146) (2.52 g, 1.0 equiv.) in THF (10 mL). The mixture was stirred for 3 hr at 0°C, carefully diluted with sat. aqueous K₂CO₃ and AcOEt. The layers were separated and the aqueous phase extracted with AcOEt (3x). The combined organic layers were dried (MgSO₄) and evaporated to give nitro compound **211** (¹H-NMR, mass spectrum). Crude compound **211** was dissolved in AcOEt (15 mL) and MeOH (15 mL), treated with 10%Pd-C (0.10 g) and stirred at RT under H₂ atmosphere (120 psi) for 12 hr. The mixture was filtered through *Celite* and the filtrate evaporated, dissolved in Et₂O, treated with HCl (g) and evaporated to give amine **212** (as the hydrochloride), which was used without further purification in the synthesis of compound **Ib-7**.

Example T

[169] The preparation of the methylenedioxy substituted isoquinoline building block **258** is described in Scheme T. This building block can be incorporated into compounds such as **Ib-39** and **I-19** using standard amide bond formation conditions (e.g., HBTU or BOPCl activation).

[170] The dialdehyde **255** was prepared in four steps from the commercially available aldehyde **250** by bromination (Harrowven et al., *Tetrahedron Lett.* 1998, 39, 6757), acetalization/formylation (Boger et al., *J. Org. Chem.* 2000, 65, 9120), and deprotection (according to Dallacker et al., *Z. Naturforsch. B Anorg. Chem. Org. Chem.* 1986, 41, 1273). Treatment of dialdehyde **255** with methyl glycinate **256** and DBU in dioxane gave the isoquinoline ester **257** that was saponified to yield the acid **258**.

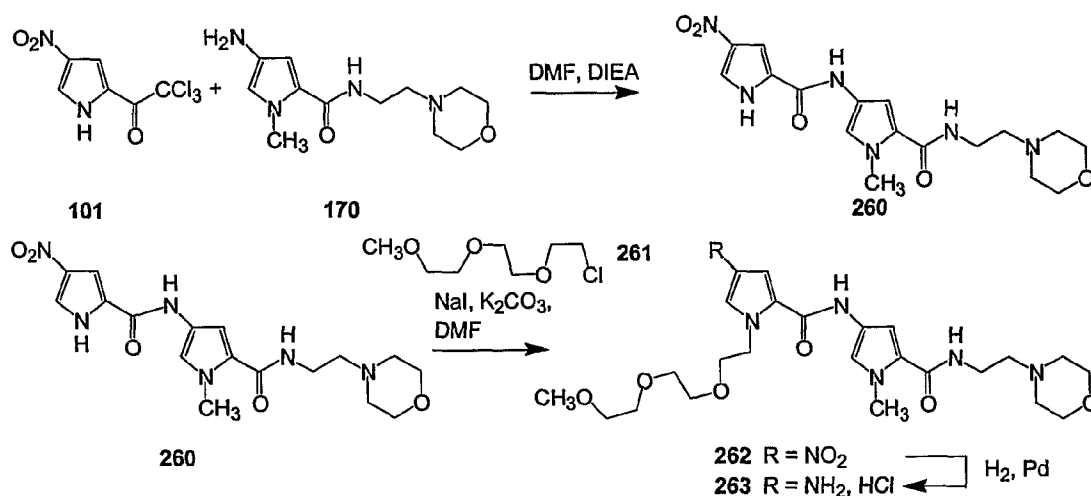
Scheme T

[171] *Synthesis of compound 257.* A mixture of dialdehyde **255** (1.48 g) and methyl glycinate hydrochloride (0.84 g, 1 equiv.) in dioxane (100 ml) and DBU (1,8-diaza-bicyclo[2.2.2]undec-7-ene, 20 ml) as refluxed for 2 h and solvent was evaporated. The residue was dissolved in AcOEt (300 ml), washed with H₂O, aqueous NH₄Cl, H₂O, brine, dried (Na₂SO₄) and evaporated. Flash chromatography of the crude product (Hex/AcOEt 4:1 to 1:1) gave compound **257** as a solid (200 mg, 10%).

[172] *Synthesis of compound 258.* A mixture of ester **257** (200 mg) in MeOH (2 ml) and 2M aqueous NaOH (10 ml) was stirred at 70°C for 12 h, cooled to 0°C, and neutralized to pH \approx 5 using 6M aqueous HCl. The resulting precipitate was collected by centrifugation, washed with H₂O (2x, each 30 ml) and Et₂O (5 ml), and dried *in vacuo* to give acid **117** (80 mg, 43%, structure confirmed by ¹H-NMR).

Example U

[173] A 4-nitro-pyrrole bearing a carboxylic ester or an amide function at position 2 can be alkylated at the ring nitrogen. The experimental details for the preparation of the pegylated pyrrole dimer **263** are described below. This dimer was used for the preparation of compound **Ib-83** (standard HBTU-mediated coupling of the acid **18** and **263**). Other nitro pyrroles, for instance the ethyl 4-nitro-pyrrole-2-carboxylate **41**, can be substituted (pegylated) analogously.

Scheme U

- [174] *Synthesis of nitro compound 260.* A mixture of trichloroketone 101 (11.32 g, 1.0 equiv.), amine 170 (15.00 g, 1.0 equiv.) in DMF (80 ml) and DIEA (20 ml) was stirred at RT for 20 h and poured into H₂O (ca. 600 ml) and sat. aqueous K₂CO₃. The solution was extracted with AcOEt (6x) and the organic layers were dried (MgSO₄) and evaporated to give nitro compound 260 as a yellow solid (structure confirmed by ¹H-NMR).
- [175] *Synthesis of pegylated nitro compound 262.* A mixture of dimer 260 (1.00 g, 1.0 equiv.), chloride 261 (3.67 g, 2.5 equiv.), NaI (576 mg, 1.5 equiv.), and K₂CO₃ (884 mg, 2.5 equiv.) in DMF (ca. 30 ml) was stirred at 65°C for 48 h, diluted with AcOEt (150 ml), and washed with sat. aqueous K₂CO₃ and H₂O (2x). The combined organic layers were dried (MgSO₄) and evaporated. Flash chromatography of the resulting oil (CH₂Cl₂: 0 to 15% MeOH) gave compound 262 as a yellow solid (785 mg, 57%, structure confirmed by ¹H-NMR and MS).
- [176] *Synthesis of amine 263.* A suspension of compound 262 (780 mg) and 10% Pd-C (200 mg) in AcOEt (36 ml) and MeOH (4 ml) was stirred at RT under H₂ (1 atm) for 22 h and filtered through *Celite*. The filtrate was treated with HCl (g) for ca. 15 seconds and evaporated to give amine 263 as a tan solid (804 mg, structure confirmed by ¹H-NMR and MS).

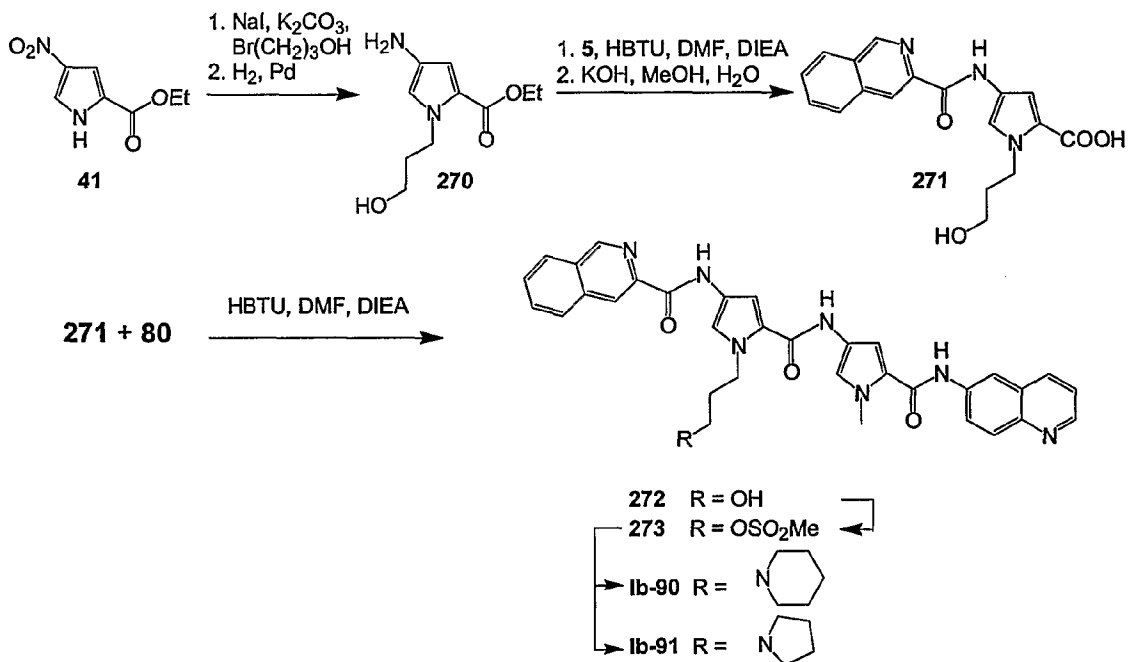
Example V

[177] This example describes the synthesis of compounds having a C-terminal quinoline, an N-terminal isoquinoline, and two internal pyrrole units, where one of the internal pyrroles bears an amino alkyl unit. This synthetic strategy allows a broad

5 variation of the nature of the amino alkyl group.

[178] Hydroxypropylated pyrrole intermediate 270 was prepared by alkylation of nitro pyrrole 41 (similarly to intermediates 42-44), followed by reduction of the nitro compound to an amine. Standard HBTU-mediated coupling of the amine and isoquinoline-3-carboxylic acid, followed by saponification afforded dimer 271. Standard

10 HBTU-coupling of dimer 271 with the previously described dimeric amine 80 gave the tetrameric alcohol 272. *In situ* mesylation of alcohol 272 and nucleophilic aromatic substitution of the mesyloxy group by an amine gave the final compounds (piperidine for Ib-90; pyrrolidine for Ib-91). Experimental details for the synthesis of Ib-90 from 272 are given below.

Scheme V

15

[179] *Synthesis of compound Ib-90.* A solution of alcohol 272 (100 mg) in DMF (1 ml, dried over 4Å molecular sieves) and DIEA (0.2 ml) was treated at RT under N₂ with methanesulfonyl chloride (freshly distilled, 0.10 ml) and stirred for 1 h. The mixture was

treated with piperidine (0.25 ml), stirred for 12 h at 60°C and diluted with 50% aqueous AcOH (10 ml). Purification by RP-HPLC gave compound **Ib-90** (structure confirmed by ¹H-NMR and MS).

In Vitro Biological Activity

5 **[180]** *In vitro* biological activity data were collected for a variety of microorganisms, including *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 27660, a methicillin resistant strain (MRSA); ATCC 13709, a methicillin sensitive strain (MSSA)); *Streptococcus pneumoniae* (ATCC 51422, a penicillin resistant strain (PRSP)), *Enterococcus faecium* (ATCC 51559, a vancomycin resistant strain (VRE)), and
10 *Staphylococcus epidermidis* (ATCC 12228). Additionally, antifungal activity data were collected for *Candida albicans* (ATCC 38247). Compounds of this invention preferably have an MIC of 4 or less against a drug resistant bacterial strain, such as one of the foregoing.

15 **[181]** Compounds according to this invention were screened for their *in vitro* activities against selected species of bacteria and fungi. The minimal inhibition concentration (MIC) of these compounds was determined using the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution assay in microtiter plates, as set forth in: (1) the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) Document M7-A4 (NCCLS, 1997); (2) the guidelines of the National Committee for
20 Clinical Laboratory Standards (NCCLS) Document M11-A4 (NCCLS, 1997); and (3) the guidelines and reference method of the National Committee for Clinical Laboratory Standards (NCCLS) Document M27-T (NCCLS, 1995). For antifungal assays, the method recommended in Murray, PR., *1995 Manual of Clinical Microbiology* (ASM Press, Washington, DC.), was employed. The results are presented in Table B below, which is
25 keyed as follows:

Key to organisms tested against:

- | | | |
|---|--------------------------------------|-------------------------------------|
| | A = <i>B. cereus</i> ATCC 11778 | B = <i>C. albicans</i> ATCC 38247 |
| | C = <i>E. faecalis</i> ATCC 29212 | D = <i>E. faecium</i> ATCC 51559 |
| | E = <i>S. aureus</i> ATCC 13709 | F = <i>S. aureus</i> ATCC 27660 |
| 5 | G = <i>S. epidermidis</i> ATCC 12228 | H = <i>S. pneumoniae</i> ATCC 49619 |
| | I = <i>S. pneumoniae</i> ATCC 51422 | |

Key to activity:

- | | | |
|----|--|---------------------|
| | +++ = MIC \leq 4 | ++ = 4 < MIC < 12 |
| | + = 12 \leq MIC \leq 32 | ND = not determined |
| 10 | >32 = preliminary data indicates MIC greater than 32 | |

Table B									
Cpd.	Organism (Minimum Inhibitory Concentration (MIC), $\mu\text{g/mL}$)								
Ref.	A	B	C	D	E	F	G	H	I
Ib-1	+++	>32	+++	ND	ND	ND	ND	+++	ND
Ib-2	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-3	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-4	+++	++	+++	+++	+++	+++	+++	+++	+++
Ib-5	+++	+++	+++	ND	+++	+++	ND	+++	ND
Ib-6	+++	>32	+++	++	+++	+++	++	+++	+++
Ib-7	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-8	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-9	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-10	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-11	++	>32	++	+	++	++	++	+++	++
Ib-12	+++	+	+++	ND	+++	+++	ND	+++	ND
Ib-13	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-14	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-15	+	>32	+	ND	++	++	ND	++	ND
Ib-16	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-17	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-18	+++	>32	+++	+++	+++	+++	++	+++	+++
Ib-19	+++	>32	+++	+	+++	+++	+++	+++	+++
Ib-20	+++	+	+++	ND	+++	+++	ND	+++	ND

Table B									
Cpd.	Organism (Minimum Inhibitory Concentration (MIC), $\mu\text{g/mL}$)								
Ref.	A	B	C	D	E	F	G	H	I
Ib-21	+++	++	+++	+++	+++	+++	+++	+++	+++
Ib-22	+++	>32	+++	ND	++	+++	ND	+++	ND
Ib-23	+++	+	+	ND	+++	+++	ND	+	ND
Ib-24	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-25	+	>32	++	ND	+	++	ND	+++	ND
Ib-26	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-27	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-28	+	>32	+	ND	++	+++	ND	+++	ND
Ib-29	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-30	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-31	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-32	++	>32	+++	ND	++	+	ND	+++	ND
Ib-33	>32	>32	>32	ND	+	+	ND	+++	ND
Ib-34	+++	+	++	ND	++	+++	ND	+++	ND
Ib-35	+++	>32	+++	ND	+++	+++	+++	+++	+++
Ib-36	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-37	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-38	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-50	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-51	+++	++	+++	++	+++	+++	++	+++	+
Ib-52	+++	>32	+++	+++	+++	+++	+++	+++	+++

Table B									
Cpd.	Organism (Minimum Inhibitory Concentration (MIC), µg/mL)								
Ref.	A	B	C	D	E	F	G	H	I
Ib-53	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-54	+++	>32	+++	+++	+++	+++	++	+++	+++
Ib-55	+++	>32	+++	++	+++	+++	+++	+++	+++
Ib-56	+++	>32	+++	++	+++	+++	+++	+++	+++
Ib-57	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-58	>32	>32	+++	ND	++	>32	ND	+++	ND
Ib-59	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-60	+++	>32	+++	ND	+++	ND	ND	+++	ND
Ib-61	>32	>32	+++	ND	+	+++	ND	+++	ND
Ib-62	+++	>32	+++	ND	++	+++	ND	+++	ND
Ib-63	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-64	+++	+++	+++	ND	+++	+++	ND	+++	ND
Ib-65	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-66	+++	+++	+++	++	+++	+++	+++	+++	+++
Ib-67	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-68	+++	+	+++	ND	+++	+++	ND	+++	ND
Ib-69	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-70	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-71	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-72	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-73	+++	+++	+++	ND	+++	+++	ND	+++	ND

Table B									
Cpd.	Organism (Minimum Inhibitory Concentration (MIC), $\mu\text{g/mL}$)								
Ref.	A	B	C	D	E	F	G	H	I
Ib-74	>32	>32	>32	ND	>32	>32	ND	>32	ND
Ib-75	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-76	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-77	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-78	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-79	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-80	+++	+++	+++	ND	+++	+++	ND	+++	ND
Ib-81	+++	+	+++	ND	+++	+++	ND	+++	ND
Ib-82	+++	+	+++	ND	+++	+++	ND	+++	ND
Ib-83	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-84	+++	+	+++	+++	+++	+++	+++	+++	+++
Ib-85	+++	+	+++	+++	+++	+++	+++	+++	+++
Ib-86	+++	++	+++	+++	+++	+++	+++	+++	+++
Ib-87	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-88	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ib-89	+++	+	+++	ND	+++	+++	ND	+++	ND
Ib-90	+++	+	+++	ND	+++	+++	ND	+++	ND
Ib-91	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-92	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ib-93	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ic-1	+++	>32	+++	+++	+++	+++	+++	+++	+++

Table B									
Cpd.	Organism (Minimum Inhibitory Concentration (MIC), $\mu\text{g/mL}$)								
Ref.	A	B	C	D	E	F	G	H	I
Ic-2	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ic-3	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ic-4	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ic-5	+	>32	+++	ND	+++	+++	ND	+++	ND
Ic-6	+++	>32	+++	ND	+++	+++	ND	+++	+++
Ic-7	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ic-8	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ic-9	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ic-10	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ic-11	>32	>32	+++	ND	+++	+++	ND	+++	ND
Ic-12	>32	>32	+++	+++	+++	+++	+++	+++	+++
Ic-13	+++	>32	+++	+++	+++	+++	+++	+++	+++
Ic-14	>32	>32	>32	ND	+++	+++	ND	+++	ND
Ic-15	>32	>32	>32	ND	+++	+++	ND	+++	ND
Ic-16	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ic-17	+++	>32	+++	ND	+++	+++	ND	+++	ND
Ic-18	+++	>32	+++	ND	+++	+++	ND	+++	ND
Id-1	+++	>32	+++	ND	+++	+++	ND	+++	ND

In Vivo Biological Data

[182] This example demonstrates *in vivo* efficacy against infection by methicillin resistant *Staphylococcus aureus* ATCC 33591, using a murine neutropenic thigh model.

[183] A *S. aureus* ATCC 33591 culture was grown to log phase overnight and diluted in phosphate buffered saline (pH 7.2) to an optical density of about 0.1 at 600 nm, giving an approximate concentration of 10^8 cfu/mL. The suspension was diluted 1:100 in phosphate buffered saline (pH 7.2) for a final concentration of 10^6 cfu/mL.

- 5 [184] Outbred female CF1 mice (approx. 20 gram body weight) were rendered neutropenic by treatment with cyclophosphamide (200 mg/kg body weight, intraperitoneal injection) at 2 and 4 days prior to inoculation. Groups of 5 mice were inoculated with 0.05 mL of the bacteria (approx. 10^6 cfu/mL) into the anterior thigh. Each group was treated intravenously two hours post infection with vehicle (phosphate
10 buffered saline) or test compound. The mice were sacrificed at either 6 or 24 hrs after treatment and thighs were collected aseptically. Each thigh was weighed, placed into sterile saline, and homogenized. The tissue homogenates were diluted appropriately for plating on agar plates. Colony counts were recorded (cfu/gram) and compared to control groups. The data are presented in Table C below:

15

Table C			
Murine Neutropenic Thigh Model			
Compound No. (Time)	Dose (mg/kg)	Colony Count (log cfu/gram)	
		Compound	Vehicle
Ib-4 (6 hr)	50	7.73	8.76
Ib-8 (6 hr)	50	5.41	8.29
Ib-55 (6 hr)	50	5.73	8.29
Ib-55 (6 hr)	25	6.54	7.74
Ib-66 (6 hr)	50	6.66	7.99
Ib-67 (6hr)	50	5.79	7.88
Ib-67 (6hr)	25	6.61	7.74
Ib-68 (6hr)	50	6.36	7.88

[185] *In vivo* efficacy was shown by a decrease in colony count (log cfu/gram of tissue) in the compound-treated animals when compared against the colony count in animals given only the vehicle.

DNA Binding

5 [186] This example illustrates the DNA binding properties of compounds of this invention using a DNase I footprinting technique. Generally, the procedure described in Dervan, WO 98/50582 (1998), was followed.

[187] Double stranded circular plasmids A and B were used to prepare double stranded DNA-binding probes containing the target sequences for the DNase I footprint titration
10 experiments.

[188] Plasmid A was prepared by hybridizing two sets of 5'-phosphorylated complementary oligonucleotides, the first set being

5' -CTAGATGCCGCTAAGTACTATGCCGCTAACTACTATGCCGCTAAT
TACTATGCCGC-3'

15 and

5' -CATAGTAATTAGCGGCATAGTAGTTAGCGGCATAGTACTTAGCGGCAT-3' ;

and the second set being

5' -TAAATACTATGCCGCTAACTAGTATGCCGCTATGCA-3'

and

20 5' -TAGCGGCATACTAGTTAGCGGCATAGTATTTAGCGG-3' ,

and ligating the resulting duplexes to the large pUC19 XbaI/PstI restriction fragment.

[189] Plasmid B was the plasmid pTrc99a, obtained from Amersham Pharmacia Biotech, Inc.

[190] The 3'-³²P end-labeled EcoRI/PvuII fragments from each plasmid were prepared
25 by digesting the plasmids with EcoRI and PvuII with simultaneous fill-in using Sequenase v. 2.0, [alpha-³²P]-deoxyadenosine-5'-triphosphate, and [alpha-³²P]-thymidine-5'-triphosphate, and isolating the cloned fragments by nondenaturing gel electrophoresis. A and G sequencing reactions were carried out as described (See Maxam and Gilbert, *Methods Enzymol.*, 1980, 65, 499-560; Iverson and Dervan, *Methods Enzymol.*, 1987, 15, 7823-7830; Sambrook et al., 1989, *Molecular Cloning*, 2nd ed., Cold
30 Spring Harbor Laboratory Press: Cold Spring Harbor, NY.) Standard methods were used for all DNA manipulations (Sambrook et al., *ibid.*)

[191] The 310 base pair dsDNA restriction fragment (SEQ ID NO. I) of Plasmid A contained a target sequence AGTACT. The 352 base pair dsDNA restriction fragment (SEQ ID NO. II) of Plasmid B contained target sequences ACAATTAT and AATTAATCAT. These fragments were used for quantitative DNase I footprinting experiments. The target sequences were selected for their identity with, or similarity to, promoter sites for bacterial genes.

[192] Quantitative DNase I footprint titration experiments were carried out as described previously (Dervan, WO 98/50582, 1998) with the following changes. All reactions were carried out in a total volume of 400 μ L, with compound stock solution or water added to 15,000 cpm radiolabeled restriction fragment affording final solution conditions of 10 mM TrisHCl, 10 mM KCl, 10 mM MgCl₂, 5 mM CaCl₂, pH 7.0 and 0.01 nM, 0.1 nM, 1.0 nM, 10.0 nM compound or no compound for reference lanes. The compounds were allowed to equilibrate at 22°C for 16 hr. Footprinting reactions were initiated with addition of 10 μ L of a DNase I stock solution (at the appropriate concentration to give ~50% intact DNA) containing 1 mM DTT and allowed to proceed for 7 min at 22°C. The reactions were stopped, ethanol precipitated, resuspended in loading buffer, heat denatured, and placed on ice as described previously (Dervan WO 98/50582, 1998). The reaction products were separated on a precast 8% polyacrylamide denaturing sequencing Castaway gel with 32 preformed wells from Stratagene in 1X TBE at 2000 V. Gels were dried according to the manufacturer and exposed to a storage phosphor screen (Molecular Dynamics). Quantitation and data analysis were carried out as described in Dervan, WO 98/50582, 1998.

[193] dsDNA binding results are provided in Table C:

Table 7 — dsDNA Binding			
Compound	Target Sequence	Dissociation Constant K_d (nM)	Target Location (Fragment/Plasmid).
Ib-19	AGTACT	50	310 bp/A
Ib-19	ACAATTAT	5	352 bp/B
Ib-56	AGTACT	<100	310 bp/A
Ib-56	AATTAATCAT	5	352 bp/B
Ib-59	AGTACT	50	310 bp/A
Ib-59	ACAATTAAT	1	352 bp/B
Ib-60	AGTACT	10	310 bp/A
Ib-60	AATTAATCAT	5	352 bp/B

SEQUENCE LISTINGS

SEQ ID NO. I (310 bp EdoRI/PvuII restriction fragment from
 5 Plasmid A; only one strand shown)
 AATTCGAGCTCGGTACCCGGGGATCCTCTAGATGCCGCTAAGTACTATGCCGCTAACTAC
 TATGCCGCTAATTACTATGCCGCTAAATACTATGCCGCTAACTAGTATGCCGCTATGCAG
 GCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCT
 CACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATG
 10 AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTCCAGTCGGGAAACCT
 GTCGTGCCAG

SEQ ID NO. II (352 bp EdoRI/PvuII restriction fragment from
 Plasmid B; only one strand shown)
 15 CTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAG
 TTAGCGGAATTGATCTGGTTTGACAGCTTATCATCGACTGCACGGTGCACCAATGCTTC
 TGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACTGCATA
 ATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCATAA
 CGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAATG
 20 TGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGACCATGGAATT

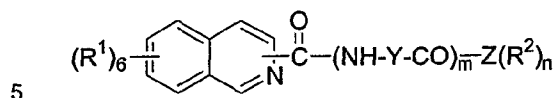
[194] The foregoing detailed description of the invention includes passages that are chiefly or exclusively concerned with particular parts or aspects of the invention. It is to be understood that this is for clarity and convenience, that a particular feature may be relevant in more than just the passage in which it is disclosed, and that the disclosure
5 herein includes all the appropriate combinations of information found in the different passages. Similarly, although the various figures and descriptions herein relate to specific embodiments of the invention, it is to be understood that where a specific feature is disclosed in the context of a particular figure or embodiment, such feature can also be used, to the extent appropriate, in the context of another figure or embodiment, in
10 combination with another feature, or in the invention in general.

[195] Further, while the present invention has been particularly described in terms of certain preferred embodiments, the invention is not limited to such preferred embodiments. Rather, the scope of the invention is defined by the appended claims.

CLAIMS

What is claimed is:

1. A compound according to the formula



and the pharmaceutically acceptable salts thereof,

wherein

each R^1 is independently H, F, Cl, Br, I, CN, OH, NO₂, NH₂, a substituted or

unsubstituted (C₁-C₁₂)alkyl group, a substituted or unsubstituted (C₁-C₁₂)alkoxy

10 group, or a substituted or unsubstituted (C₁-C₁₂)heteroalkyl group;

m is an integer from 1 to 25, inclusive;

each Y is independently a branched or unbranched, substituted or unsubstituted (C₁-

C₅)alkylene group or a substituted or unsubstituted, aromatic or heteroaromatic

ring system, wherein the ring system has a 5- or 6-member aromatic or

15 heteroaromatic rings or fused 6,6 or 6,5 aromatic or heteroaromatic rings, with the

proviso that at least one Y is a substituted or unsubstituted aromatic or

heteroaromatic ring system;

Z is either O or N;

n is 1 if Z is O and 2 if Z is N; and

20 each R^2 is independently H, a substituted or unsubstituted (C₁-C₁₂)alkyl group, or a

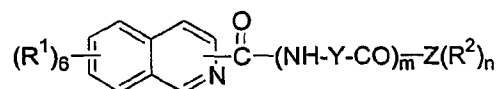
substituted or unsubstituted (C₁-C₁₂)heteroalkyl group;

the compound having a basic group having a pK_b of 12 or less or a quaternized nitrogen

group.

- 25 2. A compound according to claim 1, wherein at least one residue Y is a 5- or 6-member heteroaromatic ring.

3. A compound of the formula



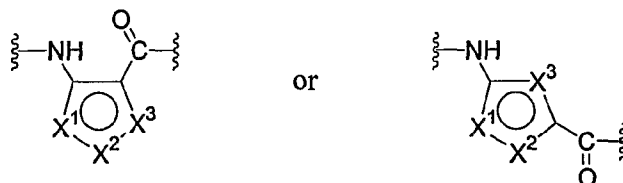
and the pharmaceutically acceptable salts thereof,

wherein

m is an integer from 1 to 25, inclusive;

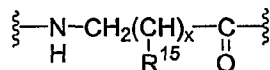
each moiety $-(NH-Y-CO)-$ is independently selected from the group consisting of

- 5 (a) moieties M^1 of the formula



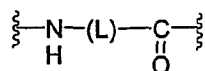
wherein one of X^1 , X^2 , and X^3 is a ring vertex selected from the group consisting of $-O-$, $-S-$, and $-NR^2-$, and the other two of X^1 , X^2 , and X^3 are ring vertices selected from the group consisting of $=N-$ and $=CR^1-$;

- 10 (b) moieties M^2 of the formula



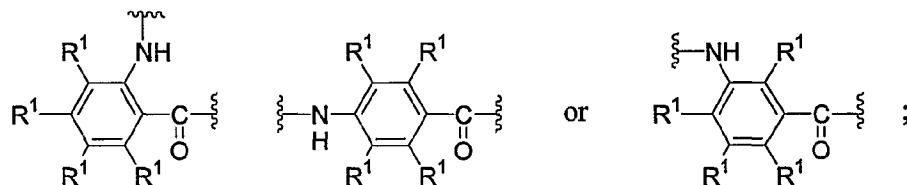
wherein x is 0 or 1 and each R^{15} is independently H, OH, NH_2 , or F;

- (c) moieties M^3 of the formula



- 15 wherein each L is independently a divalent moiety separating $-NH-$ and $-(C=O)-$ by 3 or 4 atoms; and

- (d) moieties M^4 of the formula

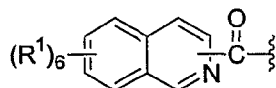


Z is O or N;

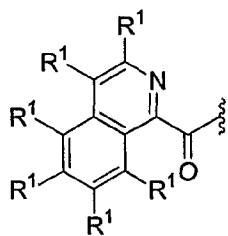
- 20 n is 1 if Z is O and 2 if Z is N;

each R^1 is independently H, F, Cl, Br, I, CN, OH, NO_2 , NH_2 , a substituted or unsubstituted (C_1-C_{12}) alkyl group, a substituted or unsubstituted (C_1-C_{12}) alkoxy group, or a substituted or unsubstituted (C_1-C_{12}) heteroalkyl group; and
 each R^2 is independently H, a substituted or unsubstituted (C_1-C_{12}) alkyl group, or a substituted or unsubstituted (C_1-C_{12}) heteroalkyl group;
 the compound having a basic group having a pK_b of 12 or less or a quaternized nitrogen group.

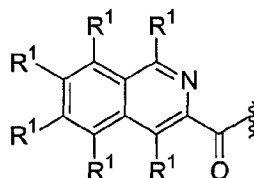
4. A compound according to claim 3, wherein the residue $Z(R^2)_n$ contains a basic group having a pK_b of 12 or less or a quaternized nitrogen group.
5. A compound according to claim 3, wherein at least one moiety $-(NH-Y-CO)-$ is M^1 .
6. A compound according to claim 3, wherein the residue



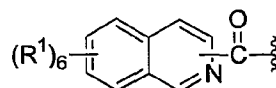
is



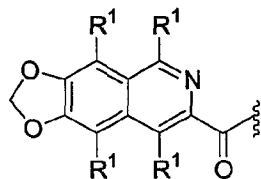
or



7. A compound according to claim 6, wherein each R^1 is H.
8. A compound according to claim 3, wherein the residue

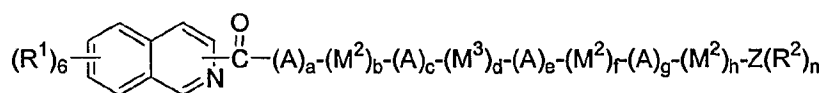


is



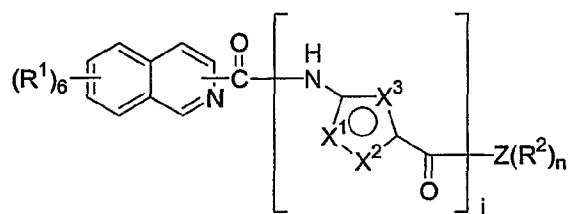
9. A compound according to claim 8, wherein each R¹ is H.

5 10. A compound according to claim 3, having the formula



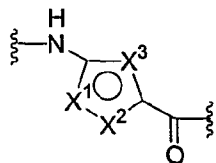
wherein M², M³, R¹, R², Z and n have the meanings assigned in claim 3; each A is independently M¹ or M⁴ as defined in claim 4; each of a, c, e, g and h is an integer independently from 0 to 5, inclusive; and each of b, d, and f is independently 0 or 1; with
10 the sum of a, c, e, and g being at least 2.

11. A compound according to claim 3, having the formula



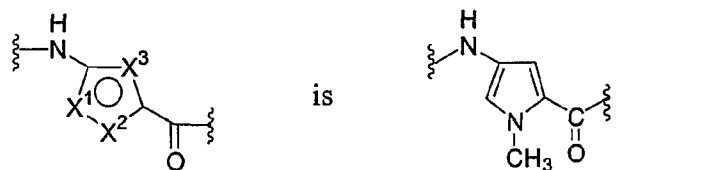
wherein X¹, X², X³, R¹, R², Z, and n have the meanings assigned in claim 3 and i is an
15 integer between 2 and 4, inclusive.

12. A compound according to claim 11, wherein a residue

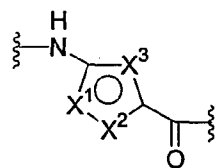


has a basic group having a pK_b of 12 or less or a quaternized nitrogen group.

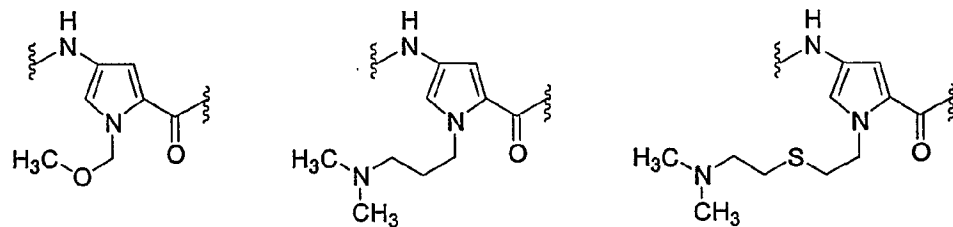
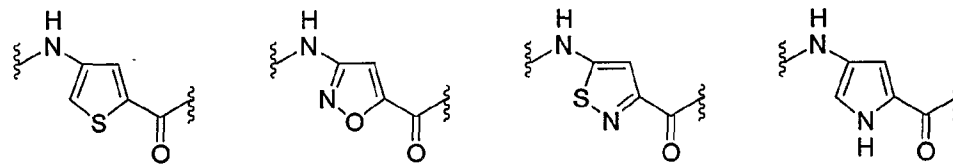
13. A compound according to claim 11, wherein each residue



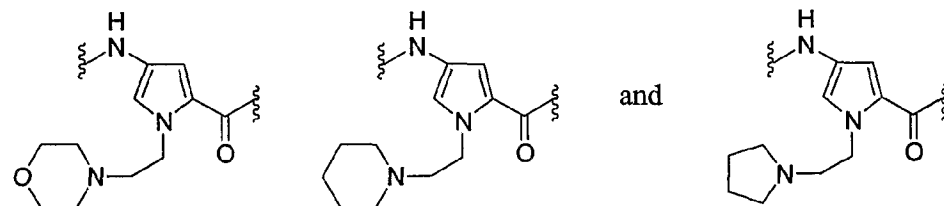
14. A compound according to claim 11, wherein at least one residue



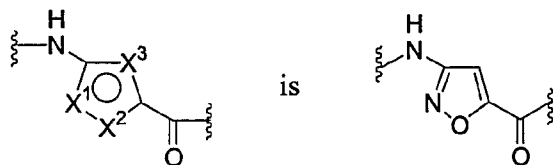
is selected from the group consisting of



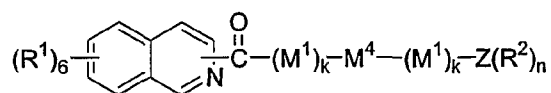
10



15. A compound according to claim 11, wherein at least one residue

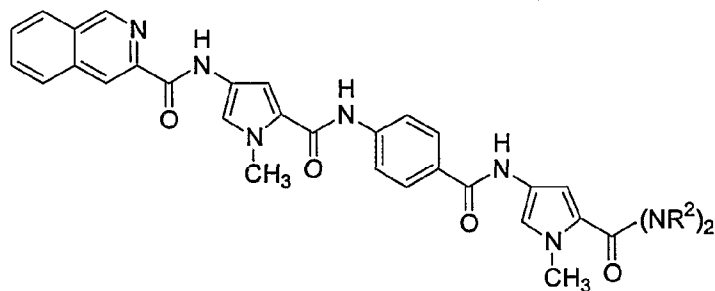


16. A compound according to claim 3, having the formula



5 wherein M^1, M^4, R^1, R^2, Z , and n are as defined in claim 3 and each k is independently an integer from 0 to 4, inclusive.

17. A compound according to claim 16, having the formula



10

18. A method of treating a bacterial infection in a mammal, comprising administering to a patient in need of such treatment an effective amount of a compound according to claim 1.

15 19. A method according to claim 18, wherein the bacterial infection is an infection by Gram-positive bacteria.

20. A method according to claim 18, wherein the bacterial infection is an infection by drug resistant bacteria.

20

21. A method according to claim 20, wherein the drug resistant bacteria is MRSA, MRSE, PRSP, or VSE.
22. A method of treating a bacterial infection in a mammal, comprising administering
5 to a patient in need of such treatment an effective amount of a compound according to claim 3.
23. A method according to claim 22, wherein the bacterial infection is an infection by Gram-positive bacteria.
- 10 24. A method according to claim 22, wherein the bacterial infection is an infection by drug resistant bacteria.
25. A method according to claim 24, wherein the drug resistant bacteria is MRSA,
15 MRSE, PRSP, or VSE.
26. The use of a compound according to claim 1 for the preparation of a medicament for the treatment of a bacterial infection in a mammal.
- 20 27. The use of a compound according to claim 3 for the preparation of a medicament for the treatment of a bacteria infection in a mammal.

Fig. 1a

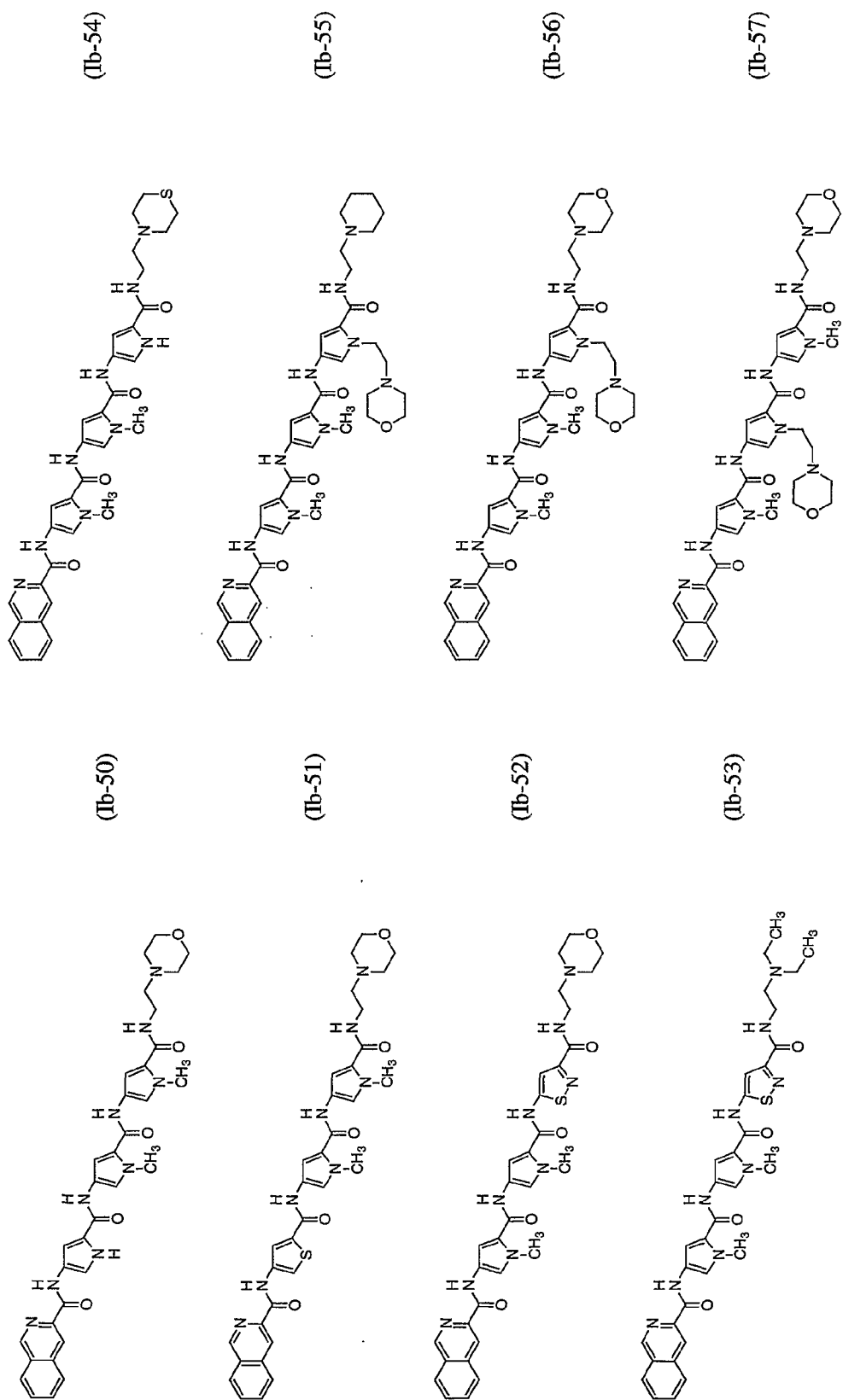


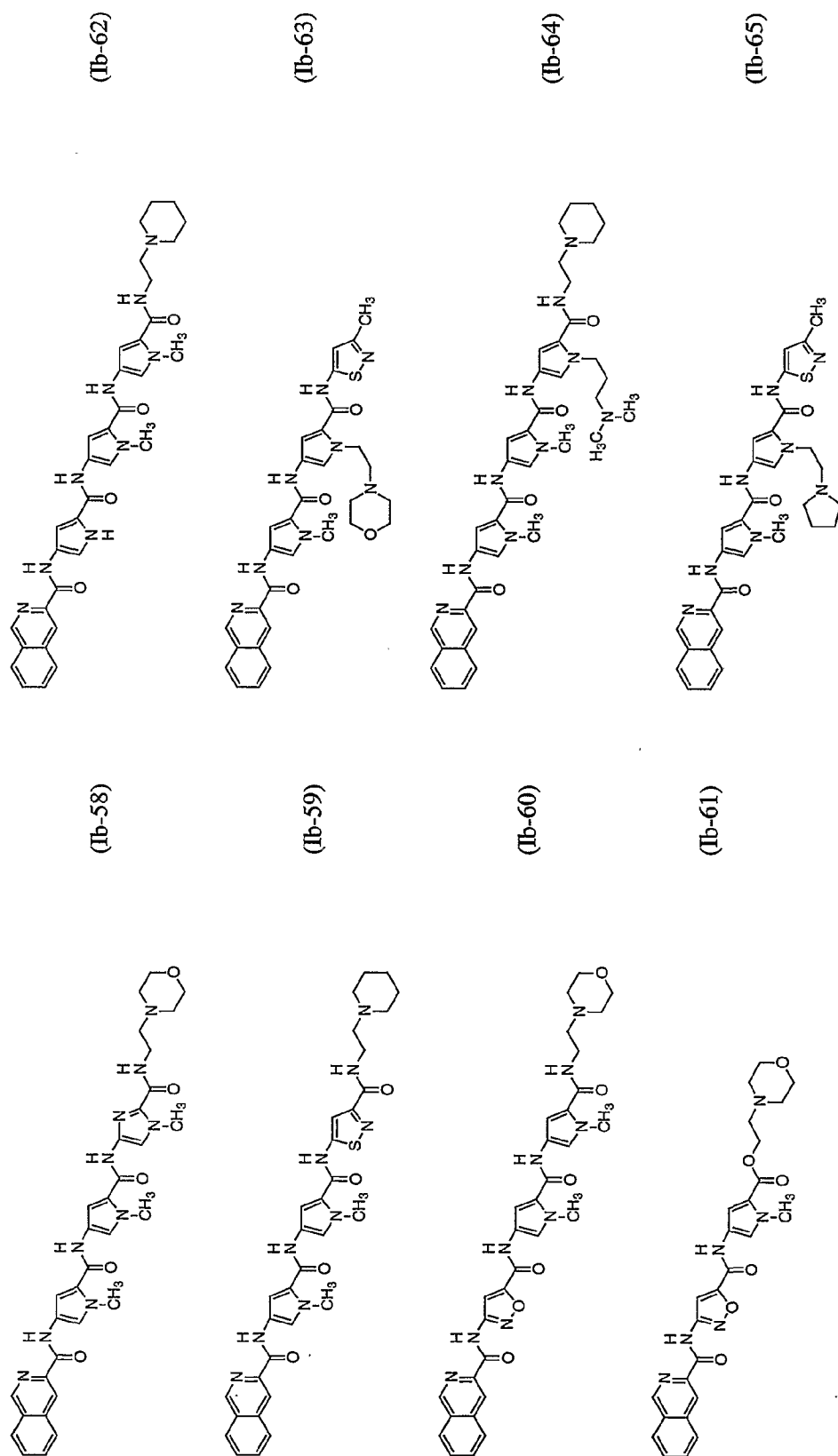
Fig. 1b

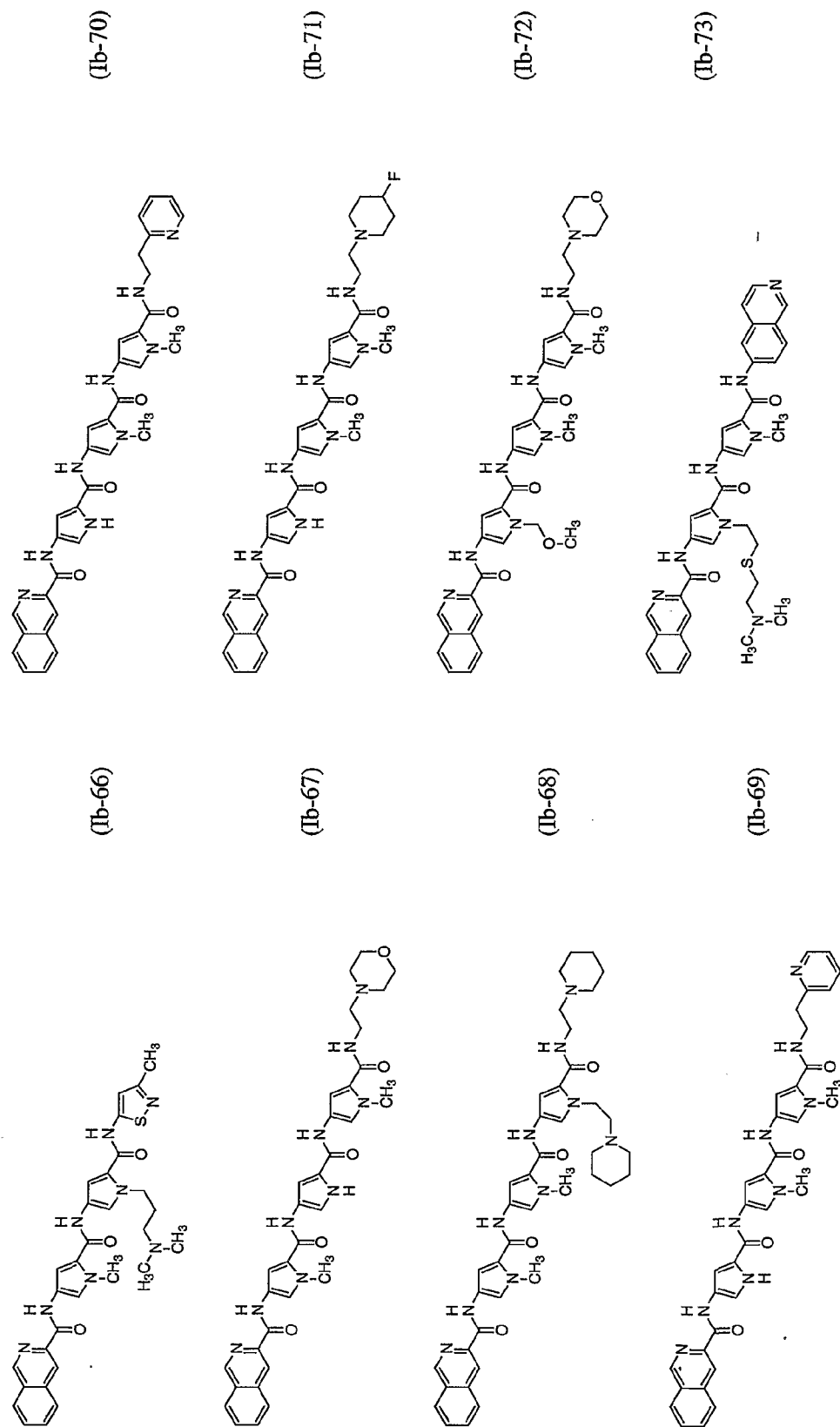
Fig. 1c

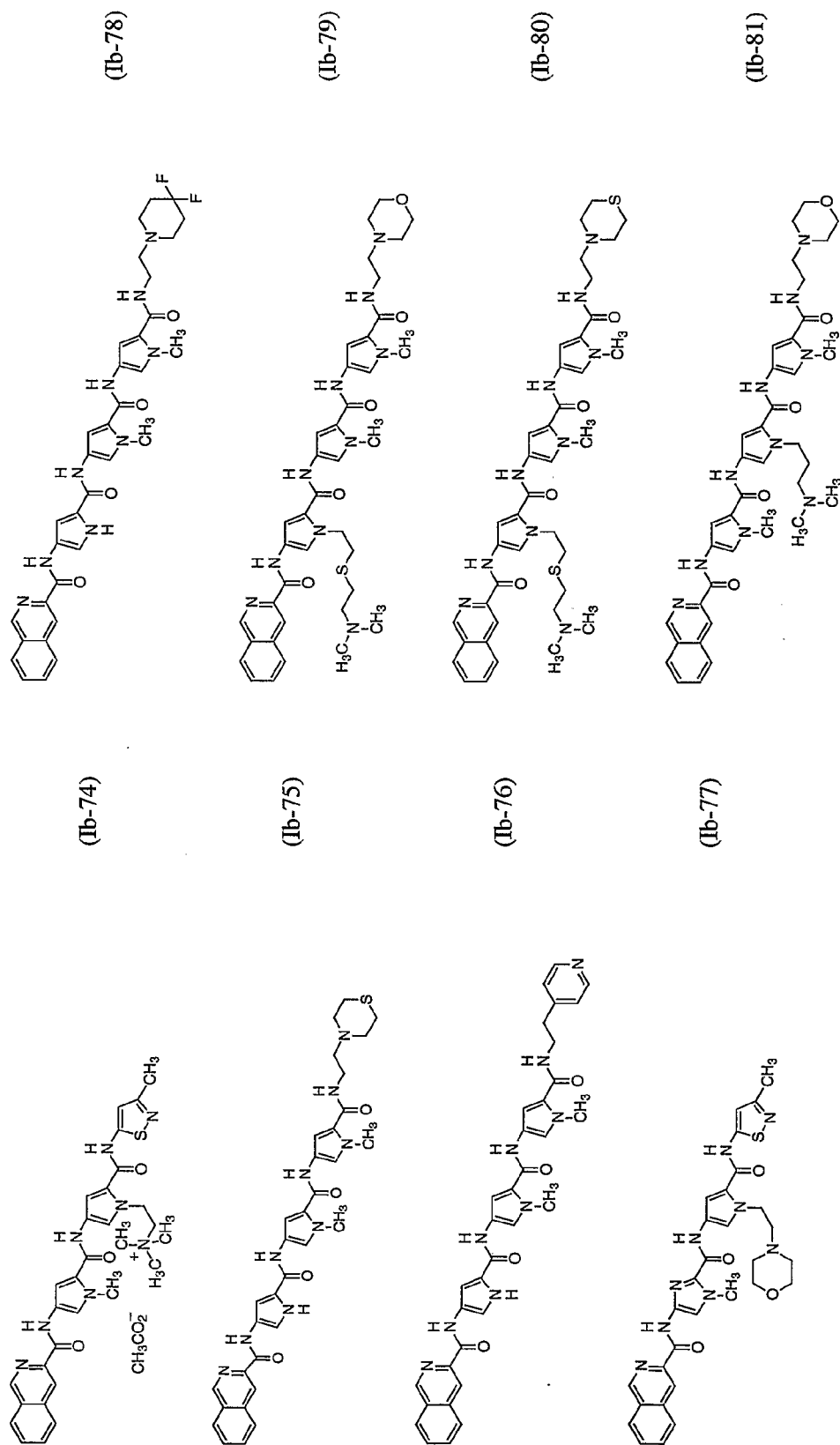
Fig. 1d

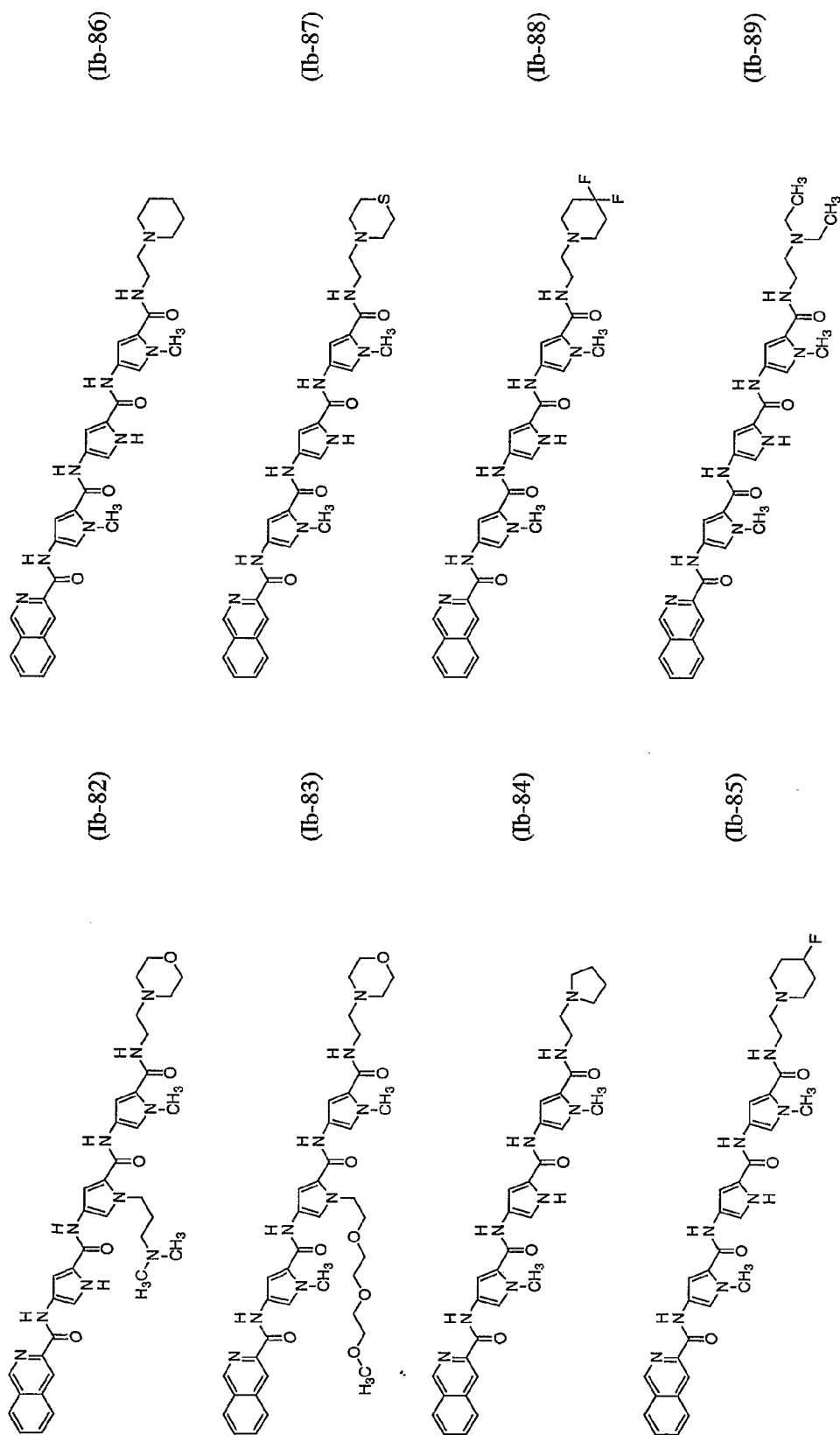
Fig. 1e

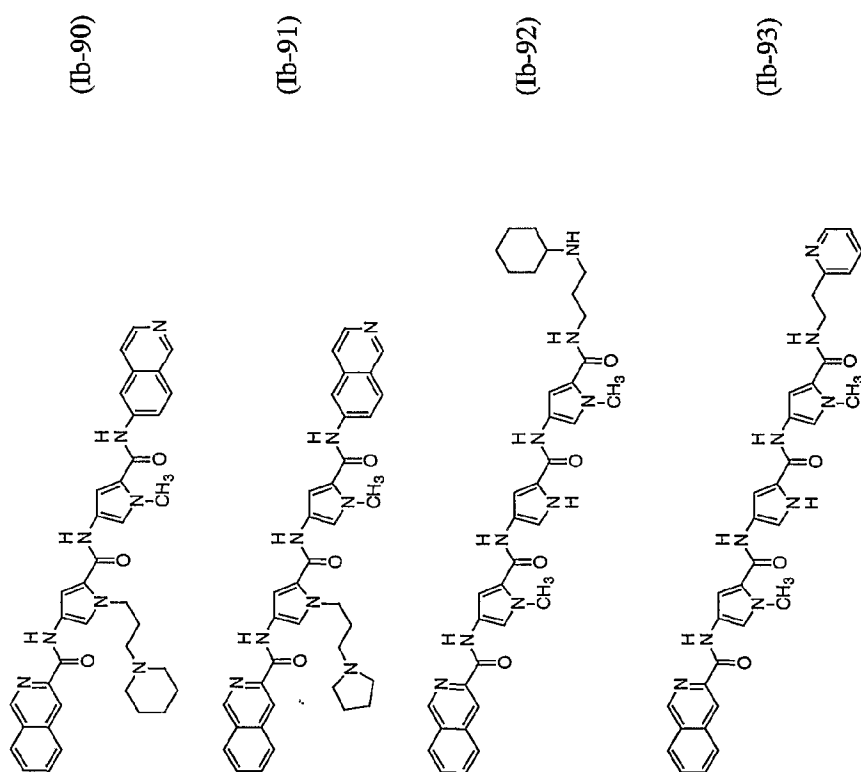
Fig. 1f

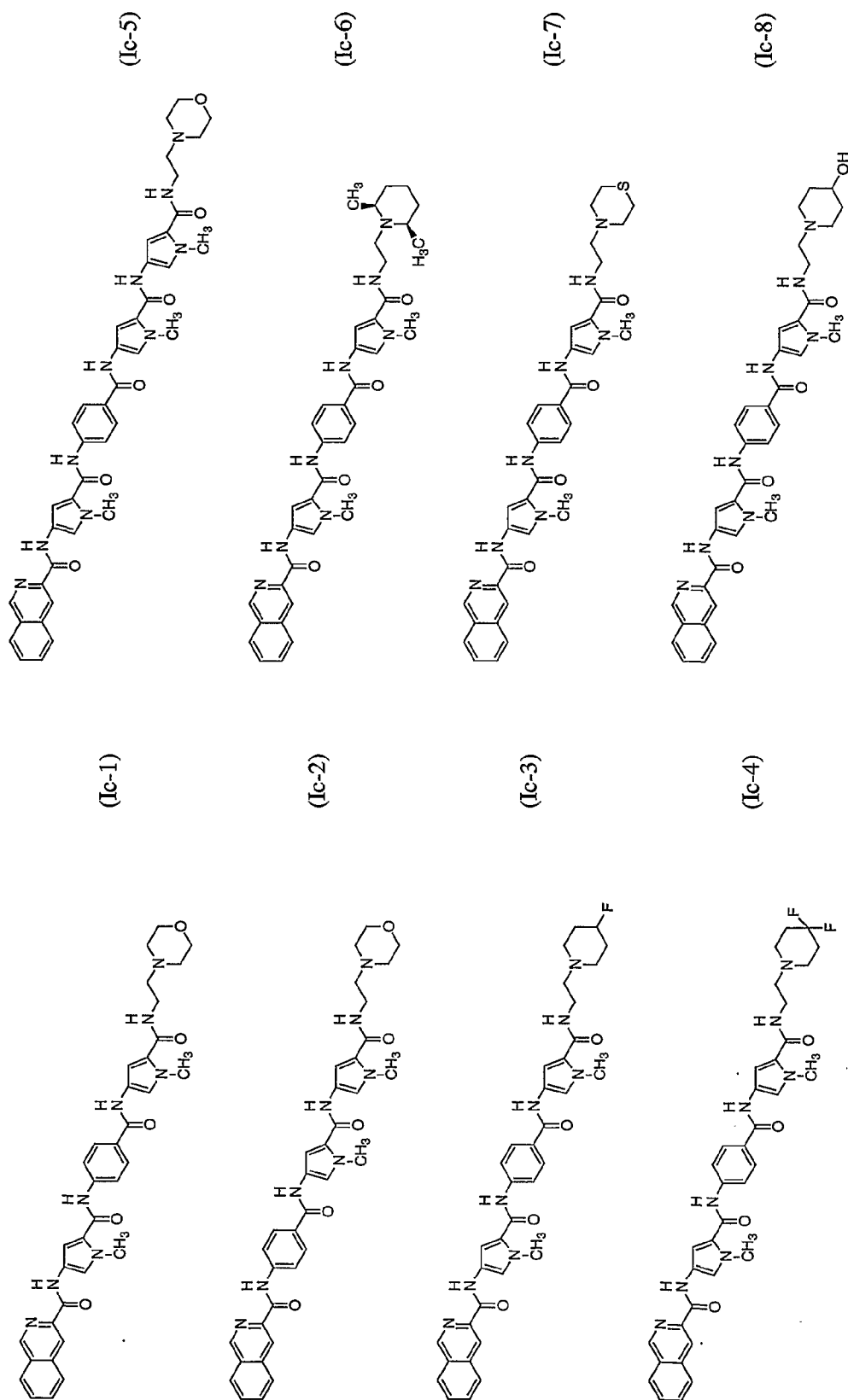
Fig. 2a

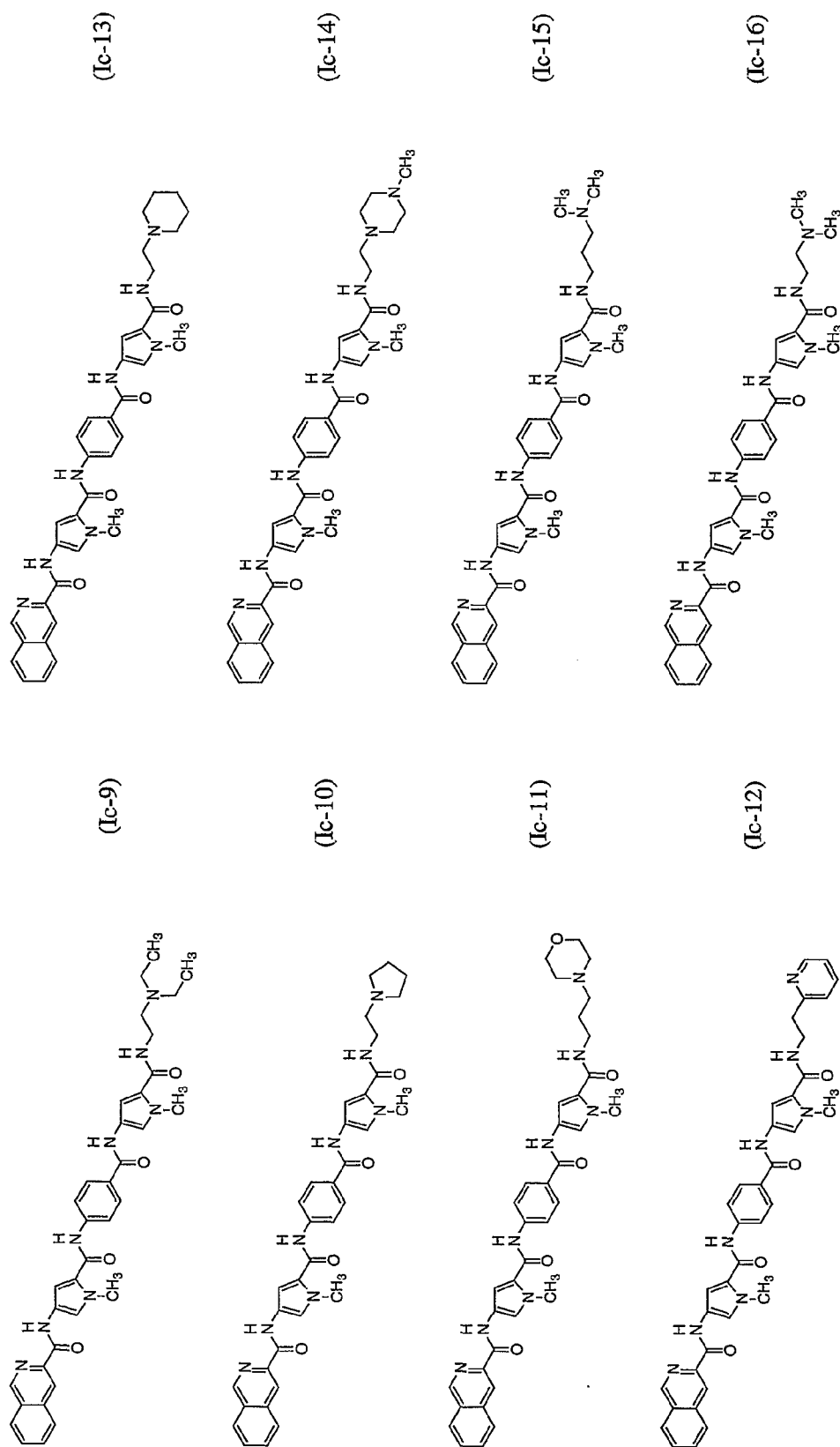
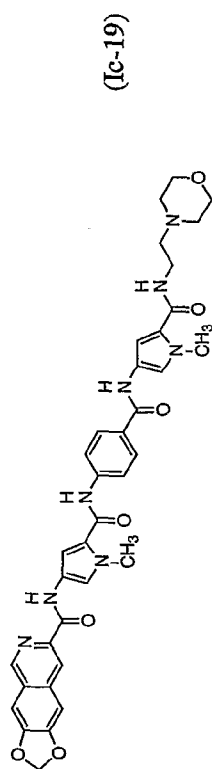
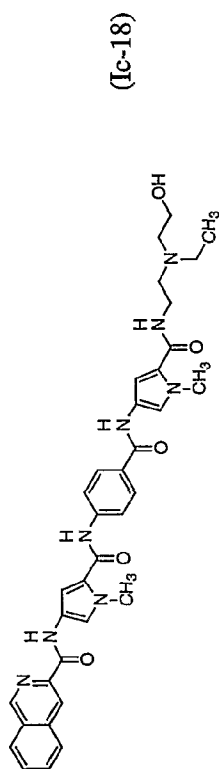
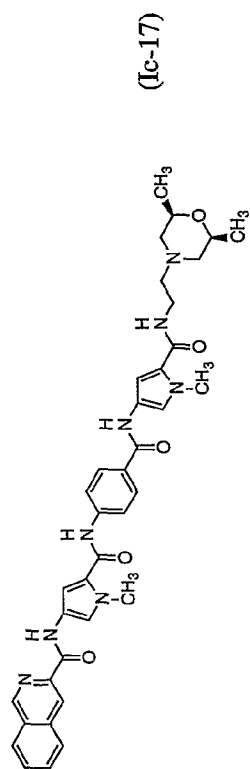
Fig. 2b

Fig. 2c



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/17954

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 217/12 ; A61K 31/47

US CL : 514/307; 546/146

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/307; 546/146

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/2351 A (LEUKOSITE, INC) 17 JULY 1997 (17.06.1997), PAGE 69, COMPOUND #2273	1 AND 3-5
X	WO 01/01615 A (YAMANOUCHI PHARMACEUTICAL CO. LTD), 29 March 2001 (29.03.2001) PAGE 29, TABLE 1, COMPOUND #36.	1 AND 3-5
X	Chemical Abstracts, Vol. 71, No. 1, 07 July 1969 (Columbus, OH USA), page 318, the Abstract No.12986e, Sen et al, 'Synthesis of Compounds Related to Reserpine Skeleton', J. Indian Chem. Soc. 1969, 46(3), 209-15 (Eng).	1 AND 3-5
A	US 5,545,640 A (BEAULIEU et al) 13 August 1996 (19.08.1996), see entire document.	1-25

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	"T"
"A" document defining the general state of the art which is not considered to be of particular relevance	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

Date of mailing of the international search report

19 September 2002 (19.09.2002)

21 NOV 2002

Name and mailing address of the ISA/US

Authorized officer

Commissioner of Patents and Trademarks
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Telephone No. 703-308-1235

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/17954

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 26 and 27
because they relate to subject matter not required to be searched by this Authority, namely:
The recitation of the term "use" renders the claims non-statutory.
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to
such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule
6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite
payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search
report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report
is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.